

# **Supplementary data S1:**

## **datasets information**

## List of markers used in this study

Gene name	Gene description	Refseq ID	Marker used in		Number of conserved positions	
			Derelle and Lang 2012	He et al. 2014	ALPHA-PROT dataset	EUBAC dataset
atp3	ATP synthase F1 subunit gamma	NP_001001973	X		84	--
cox11	Protein involved in heme biosynthesis	NP_004366	X		110	--
grpE	Mitochondrial cochaperone grpE	NP_079472	X		60	--
HslVU	Heat shock protein HslVU	XP_636662	X		264	--
Hsp78	Mitochondrial Hsp 78	XP_636444	X		544	--
mHsp60	Mitochondrial Hsp 60 kDa	NP_002147	X		434	--
mrl3	Mitochondrial ribosomal protein L3	NP_009139	X		95	--
mtif2	Translation initiation factor IF-2	NP_002444	X		146	--
nad7	NADH dehydrogenase subunit 7	NP_004541	X		358	--
nad8	NADH dehydrogenase subunit 8	NP_002487	X		119	--
nad10	NADH dehydrogenase subunit 10	NP_077718	X		133	--
nad11	NADH dehydrogenase subunit 11	NP_004997	X		290	--
ndufv1	NADH dehydrogenase flavoprotein 1	NP_001159574	X		397	--
ndufv2	NADH dehydrogenase flavoprotein 2	NP_066552	X		75	--
odpB	Pyruvate dehydrogenase E1 component, beta sub.	NP_000916	X		276	--
sdh2	Succinate dehydrogenase subunit 2	NP_002991	X		177	--
CLPP	Caseinolytic peptidase, proteolytic subunit	NP_006003			168	--
ETFDH	Electron-transferring-flavoprotein dehydrogenase	NP_004444			320	--
ndufaf5	NADH dehydrogenase complex I, assembly factor 5	NP_077025			102	--
NifU	Iron-sulfur cluster scaffold homolog, mitochondrial	NP_056515			104	--
PCCA	Propionyl CoA carboxylase, alpha polypeptide	NP_000273			388	--
PCCB	Propionyl CoA carboxylase, beta polypeptide	NP_000523			424	--
PET112	glutamyl-tRNA amidotransferase subunit B	NP_004555			198	--
TyPA	Tyrosine phosphorylated protein A	XP_643254			313	--
atp1	ATP synthase F1 subunit alpha	NP_001001937	X	X	409	
atp2	ATP synthase F1 subunit beta	NP_001677	X	X	428	
cytc1	Cytochrome c1	NP_001907	X	X	134	
DLDH	Dihydrolipoyl dehydrogenase	NP_000099	X	X	313	
DLST	Dihydrolipoamide S-succinyltransferase	NP_001924	X	X	234	
etfb	Electron transfer flavoprotein, beta subunit	NP_001976	X	X	141	
mHsp70	Mitochondrial Hsp 70 kDa	NP_004125	X	X	492	
nsf1	Cysteine desulfurase, mitochondrial	NP_066923	X	X	290	
odpA	Pyruvate dehydrogenase E1 component, alpha sub.	NP_005381	X	X	199	
sdh1	Succinate dehydrogenase subunit 1	NP_004159	X	X	484	
SUCLG1	SuccinylCoA ligase, alpha subunit	NP_003840	X	X	248	
tufA	Translation elongation factor Tu	NP_003312	X	X	324	
ndufa9	NADH dehydrogenase 1 alpha subcomplex, 9	NP_004993		X	82	
UQCRC1	Mitochondrial-processing peptidase subunit beta	NP_004270		X	198	
UQCRCFS1	Cytochrome b-c1 complex subunit Rieske	NP_005994		X	86	
prot_10	ATP-dependent molecular chaperone HSC82	NP_523899		X	--	495
prot_11	Cytosolic Fe-S cluster assembly NBP35	NP_002475		X	--	122
prot_13	Aspartyl aminopeptidase 4	NP_036232		X	--	200
prot_14	2-oxoglutarate dehydrogenase	NP_060715		X	--	433
prot_15	Ribonucleoside-diphosphate reductase large chain 1	NP_001024		X	--	551
prot_17	Protoheme IX farnesyltransferase	NP_001294		X	--	97
prot_18	Respiratory chain complexes assembly YTA12	NP_006787		X	--	330
prot_21	Adenylate kinase 1	NP_037543		X	--	145
prot_24	ATP-dependent RNA helicase PRP43	NP_001349		X	--	427
prot_25	Dihydrolipoyllysine-residue acetyltransferase	NP_001922		X	--	140
prot_26	methylcrotonoyl-CoA carboxylase beta chain	NP_071415		X	--	361
prot_32	Aminomethyltransferase	NP_000472		X	--	121
prot_33	Glycerol-3-phosphate dehydrogenase	NP_001076581		X	--	187
prot_34	Branched-chain-amino-acid aminotransferase	NP_001171564		X	--	137
prot_35	Protein SCO2	NP_004580	X	X	--	67
prot_36	Isocitrate dehydrogenase subunit 2	NP_005521		X	--	263
prot_37	Iron-sulfur clusters transporter ATM1	NP_004290		X	--	342
prot_4	Elongation factor G1	NP_079272		X	--	404
prot_5	S-adenosylmethionine synthase 1	NP_005902		X	--	280
prot_6	Pyruvate kinase 1	XP_639190		X	--	243
prot_7	Threonyl tRNA synthetase	NP_689508		X	--	305
prot_9	Aspartate aminotransferase	NP_002071		X	--	250

## List of species used in this study

			% data	
Species		Origin of data	ALPHA-PROT dataset	EUBAC dataset
EUKARYOTA	<i>Karenia brevis</i>	CAMERA 2.0 portal	51.7	66.9
	<i>Lingulodinium polyedrum</i>	CAMERA 2.0 portal	50.6	33.6
	<i>Perkinsus marinus</i>	NCBI Refseq	53.8	76.5
	<i>Plasmodium falciparum</i>	NCBI Refseq	46.0	73.7
	<i>Toxoplasma gondii</i>	NCBI Refseq	56.8	90.6
	<i>Oxytricha trifallax</i>	NCBI Refseq	76.4	84.3
	<i>Paramecium tetraurelia</i>	NCBI Refseq	74.5	94.2
	<i>Platyophrya macrostoma</i>	CAMERA 2.0 portal	63.8	38.1
	<i>Tetrahymena thermophila</i>	NCBI Refseq	73.7	93.3
	<i>Acanthamoeba castellanii</i>	NCBI Refseq	93.6	81.8
	<i>Dictyostelium discoideum</i>	NCBI Refseq	93.5	95.8
	<i>Filamoeba nolandi</i>	CAMERA 2.0 portal	77.4	84.4
	<i>Physarum polycephalum</i>	NCBI Refseq	71.0	60.3
	<i>Polysphondylium pallidum</i>	NCBI Refseq	92.4	94.0
	<i>Stereomyxa ramosa</i>	CAMERA 2.0 portal	77.1	85.6
	<i>Collodictyon triciliatum</i>	Zhao <i>et al.</i> 2013 dataset	62.0	36.2
	<i>Thecamonas trahens</i>	BROAD Institute	76.6	91.0
	<i>Cryptomonas paramecium</i>	CAMERA 2.0 portal	68.4	76.8
	<i>Goniomonas avonlea</i>	<b>new data</b>	77.1	71.1
	<i>Guillardia theta</i>	JGI	83.8	88.1
	<i>Acrasis kona</i>	He <i>et al.</i> 2014 dataset	35.5	82.2
	<i>Euteptiella gymnastica</i>	CAMERA 2.0 portal	71.1	35.0
	<i>Leishmania major</i>	NCBI Refseq	79.3	80.9
	<i>Naegleria gruberi</i>	NCBI Refseq	89.4	93.9
	<i>Andalucia godoyi</i>	<b>new data</b>	96.4	99.0
	<i>Jakoba libera</i>	TBestDB + NCBI	40.6	34.1
	<i>Reclimononas americana</i>	TBestDB + NCBI	44.6	44.7
	<i>Secularomonas ecuadorensis</i>	TBestDB + NCBI	37.6	48.5
	<i>Malawimonas californiana</i>	<b>new data</b>	95.1	87.3
	<i>Malawimonas jakobiformis</i>	<b>new data</b>	95.7	84.5
	<i>Batrachochytrium dendrobatidis</i>	JGI	91.9	96.5
	<i>Gonapodya prolifera</i>	JGI	82.4	84.2
	<i>Mortierella verticillata</i>	BROAD Institute	93.9	96.5
	<i>Phanerochaete chrysosporium</i>	JGI	80.0	36.3
	<i>Rhizopus oryzae</i>	BROAD Institute	93.6	97.2
	<i>Spizellomyces punctatus</i>	JGI	93.7	97.5
	<i>Tuber melanosporum</i>	NCBI Refseq	80.6	37.7
	<i>Ustilago maydis</i>	NCBI Refseq	76.4	92.2
	<i>Yarrowia lipolytica</i>	NCBI Refseq	83.5	97.5
	<i>Acropora digitifera</i>	<a href="http://marinegenomics.oist.jp">http://marinegenomics.oist.jp</a>	71.5	86.0
	<i>Amphimedon queenslandica</i>	NCBI Refseq	84.7	91.7
	<i>Capsaspora owczarzaki</i>	BROAD Institute	91.5	91.6
	<i>Ephydatia muelleri</i>	Compagen	86.1	96.6
	<i>Homo sapiens</i>	NCBI Refseq	88.3	97.5
	<i>Lottia gigantea</i>	JGI	84.4	95.0
	<i>Monosiga brevicollis</i>	NCBI Refseq	84.7	78.1
	<i>Monosiga ovata</i>	NCBI dB EST	67.2	62.2
	<i>Nematostella vectensis</i>	NCBI Refseq	85.7	93.4
	<i>Salpingoeca rosetta</i>	BROAD Institute	86.8	92.1
	<i>Bigelowiella natans</i>	JGI	91.2	82.8
	<i>Chondrus crispus</i>	NCBI Refseq	78.0	92.6
	<i>Galdieria sulphuraria</i>	NCBI Refseq	92.8	91.8
	<i>Aplanochytrium kerguelense</i>	JGI	94.0	88.6
	<i>Schizochytrium aggregatum</i>	JGI	91.0	92.4
	<i>Phytophthora infestans</i>	NCBI Refseq	94.1	91.3
	<i>Saprolegnia parasitica</i>	BROAD Institute	94.9	92.2
	<i>Bolidomonas pacifica</i>	CAMERA 2.0 portal	80.8	93.9
	<i>Ectocarpus siliculosus</i>	NCBI Refseq	87.3	86.1
	<i>Thalassiosira pseudonana</i>	NCBI Refseq	95.8	87.1
	<i>Heterosigma akashiwo</i>	CAMERA 2.0 portal	76.2	77.3
	<i>Chlorella variabilis</i>	JGI	74.6	89.5

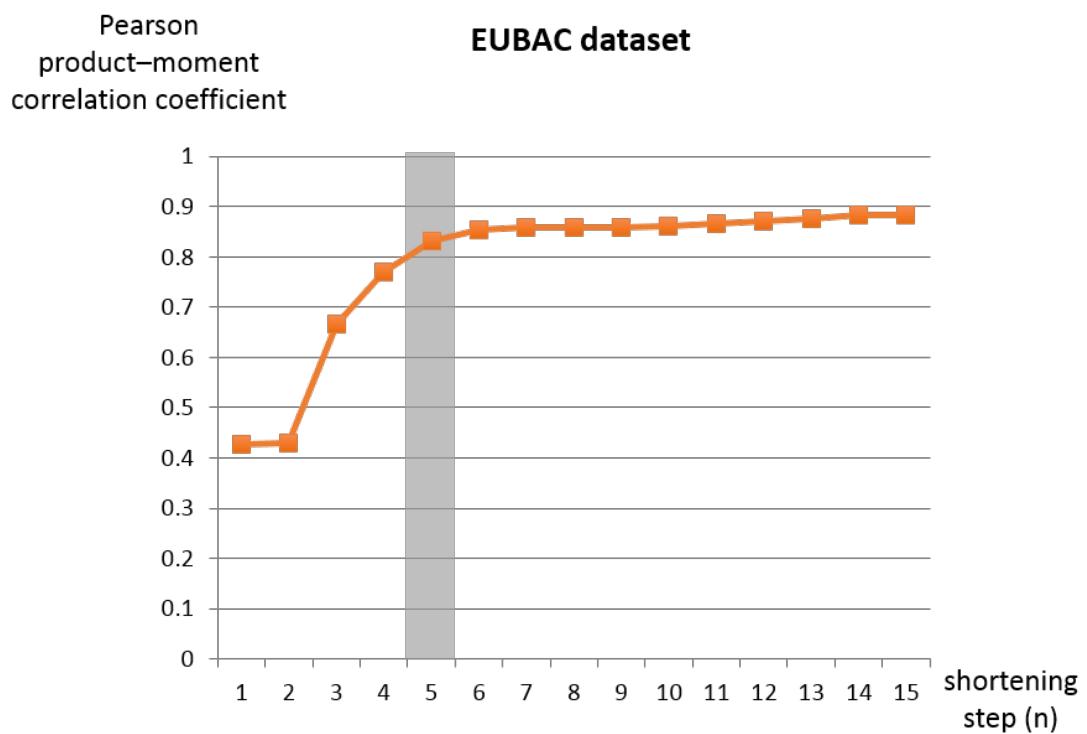
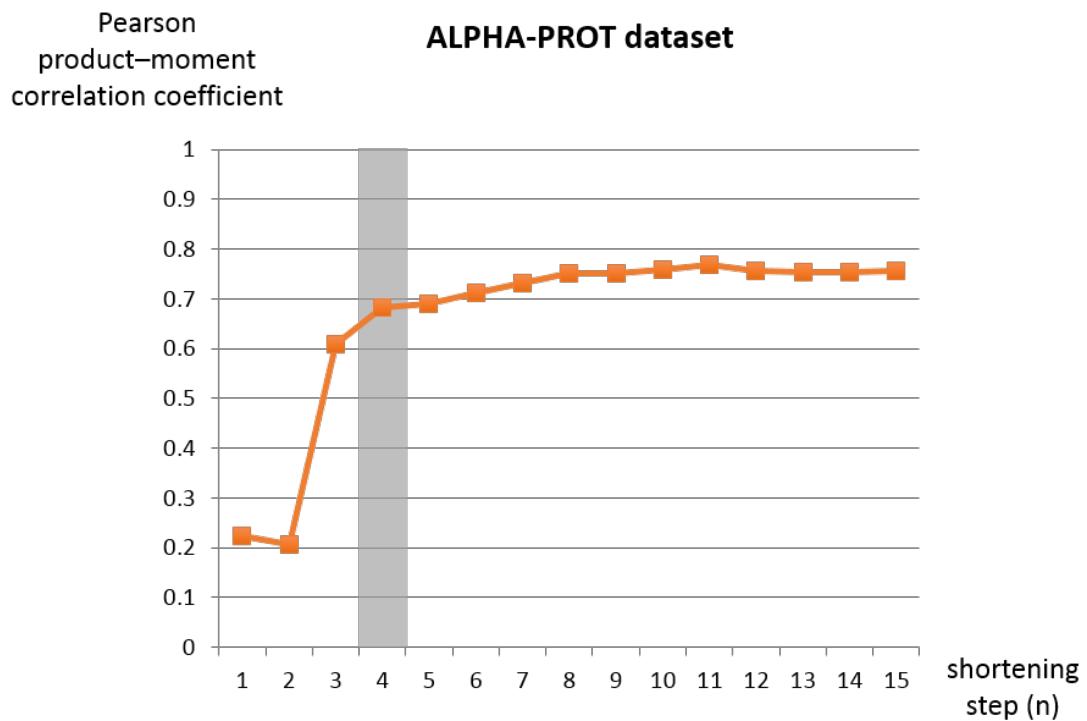
	Micromonas sp	NCBI Refseq	79.0	89.1
	Ostreococcus lucimarinus	JGI	71.7	88.1
	Tetraselmis astigmatica	CAMERA 2.0 portal	75.6	86.5
	Arabidopsis thaliana	NCBI Refseq	74.9	94.4
	Oryza sativa	NCBI Refseq	80.8	97.1
	Physcomitrella patens	NCBI Refseq	83.7	99.9
ALPHA-PROTEOBACTERIA OUTGROUP	Maricaulis maris	NCBI Refseq	88.9	--
	Parvularcula bermudensis	NCBI Refseq	84.9	--
	Parvibaculum lavamentivorans	NCBI Refseq	96.6	--
	Starkeya novella	NCBI Refseq	96.7	--
	Azospirillum sp	NCBI Refseq	93.6	--
	Rhodospirillum centenum	NCBI Refseq	90.3	--
	Roseomonas cervicalis	NCBI Refseq	91.1	--
	Granulibacter bethesdensis	NCBI Refseq	87.8	--
	Magnetospirillum magneticum	NCBI Refseq	99.8	--
	Rhodospirillum rubrum	NCBI Refseq	95.7	--
EUBACTERIA OUTGROUP	Magnetococcus sp	NCBI Refseq	73.5	--
	Solibacter usitatus	NCBI Refseq	--	61.1
	Agrobacterium tumefaciens	NCBI Refseq	--	87.0
	Magnetospirillum magneticum	NCBI Refseq	--	80.1
	Pelagibacter ubique	NCBI Refseq	--	52.7
	Puniceispirillum marinum	NCBI Refseq	--	67.3
	Rhodopirellula baltica	NCBI Refseq	--	81.7
	Rickettsia conorii	NCBI Refseq	--	68.3
	Burkholderia ambifaria	NCBI Refseq	--	86.9
	Desulfovibrio vulgaris	NCBI Refseq	--	59.9
	Geobacter metallireducens	NCBI Refseq	--	68.0
	Escherichia coli	NCBI Refseq	--	79.2
	Myxococcus xanthus	NCBI Refseq	--	72.2
	Planctomyces limnophilus	NCBI Refseq	--	70.2
			AVERAGE	79.8 %
				79.8 %

### **Removal of fast evolving sites**

We performed a removal of fast evolving sites from both matrices using a tree independent approach (i.e. the evolutionary rate of each sites were estimated without reference phylogenetic tree) and with a stopping criterion derived from Goremykin *et al.* 2010.

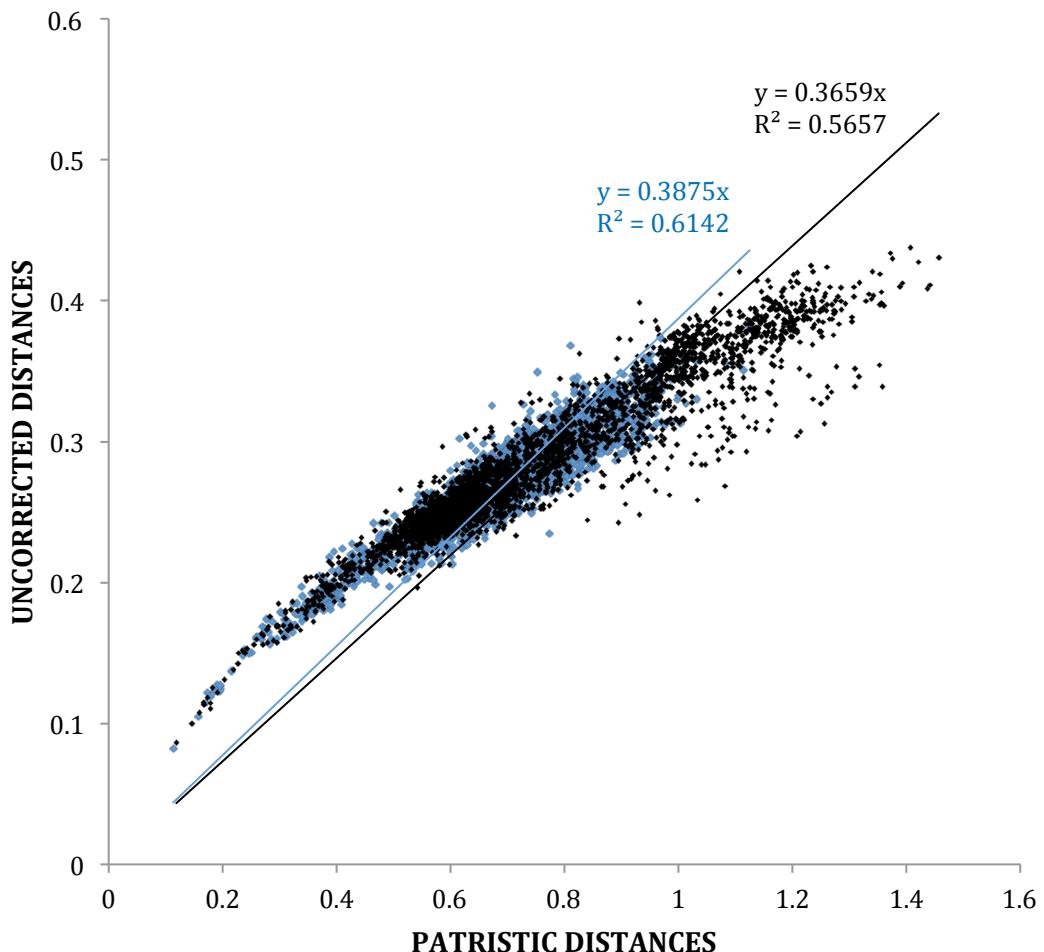
In practice, we applied the following successive steps:

- \_ Evolutionary rates of each sites were estimated by their similarity score calculated by trimAl (Capella-Gutiérrez, Silla-Martínez and Gabaldón 2009).
- \_ Fast-evolving sites were repeatedly removed from the original matrices with a 1% increment. At each shortening step (n), 2 partitions were created: the shortened alignment (referred to hereafter as  $A_n$ ) and all deleted positions (referred to hereafter as  $B_n$ ).
- \_ Species that have more than 30% of missing data in the two original matrices were removed from all  $A_n$  and  $B_n$  alignments (because missing data affect corrected distances calculated from short alignments).
- \_ The best evolutionary model were estimated using ProtTest (option '-S 1'; Darriba *et al.* 2011) for each  $A_n$  and  $B_n$  alignments
- \_ Pair-wise ML distances were calculated from all  $A_n$  and  $B_n$  alignments using RAxML (option '-f x'; Stamatakis 2006) under their corresponding evolutionary models.
- \_ At each shortening step, pair-wise ML distances inferred from  $A_n$  and  $B_n$  alignments were compared by calculating the Pearson product-moment correlation coefficient. Results are shown in the two graphics below.
- \_ For each matrices, the best shortening step corresponds to the step where the Pearson product-moment correlation coefficient reach a plateau (gray bars in the two graphics below)



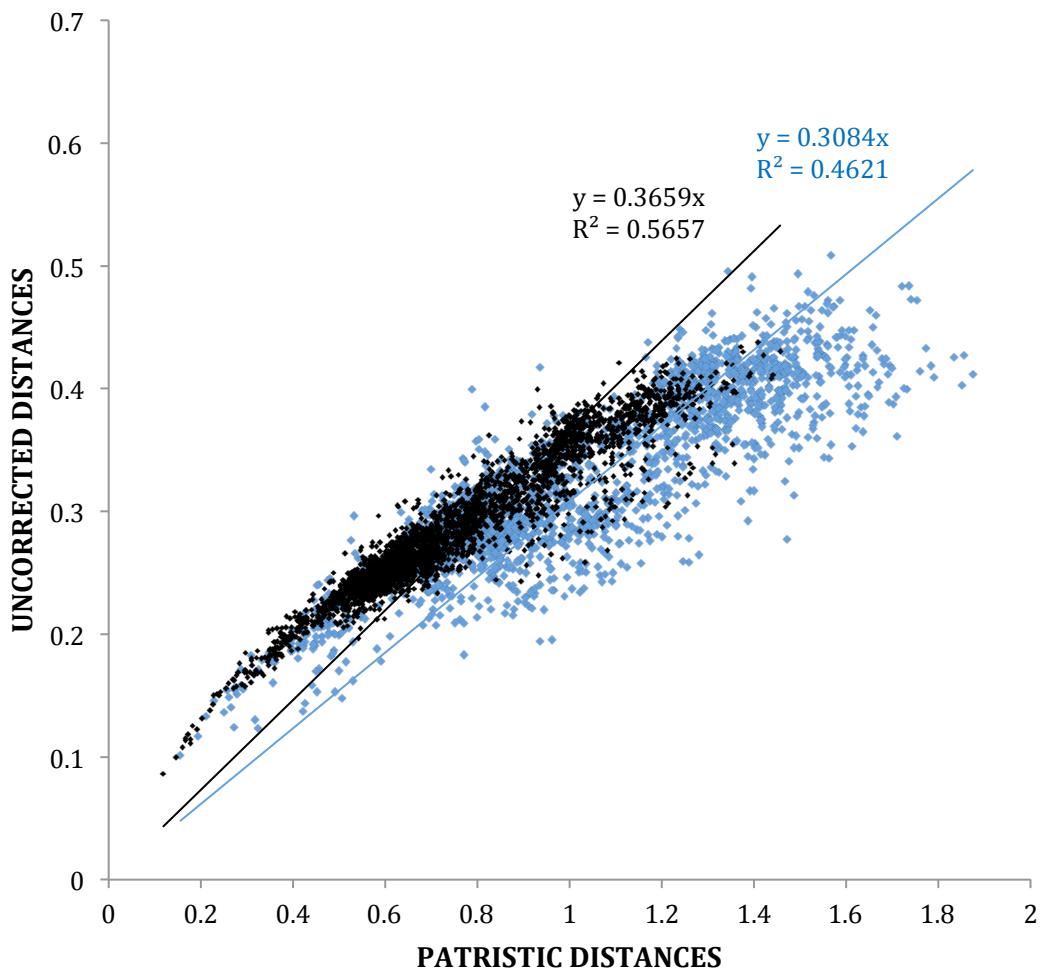
**Saturation plot of the two datasets analyzed in this study**

Saturation plots of the ALPHA-PROT (blue dots and blue regression line) and EUBAC (black dots and black regression line) datasets. See Supplementary Material for method details.



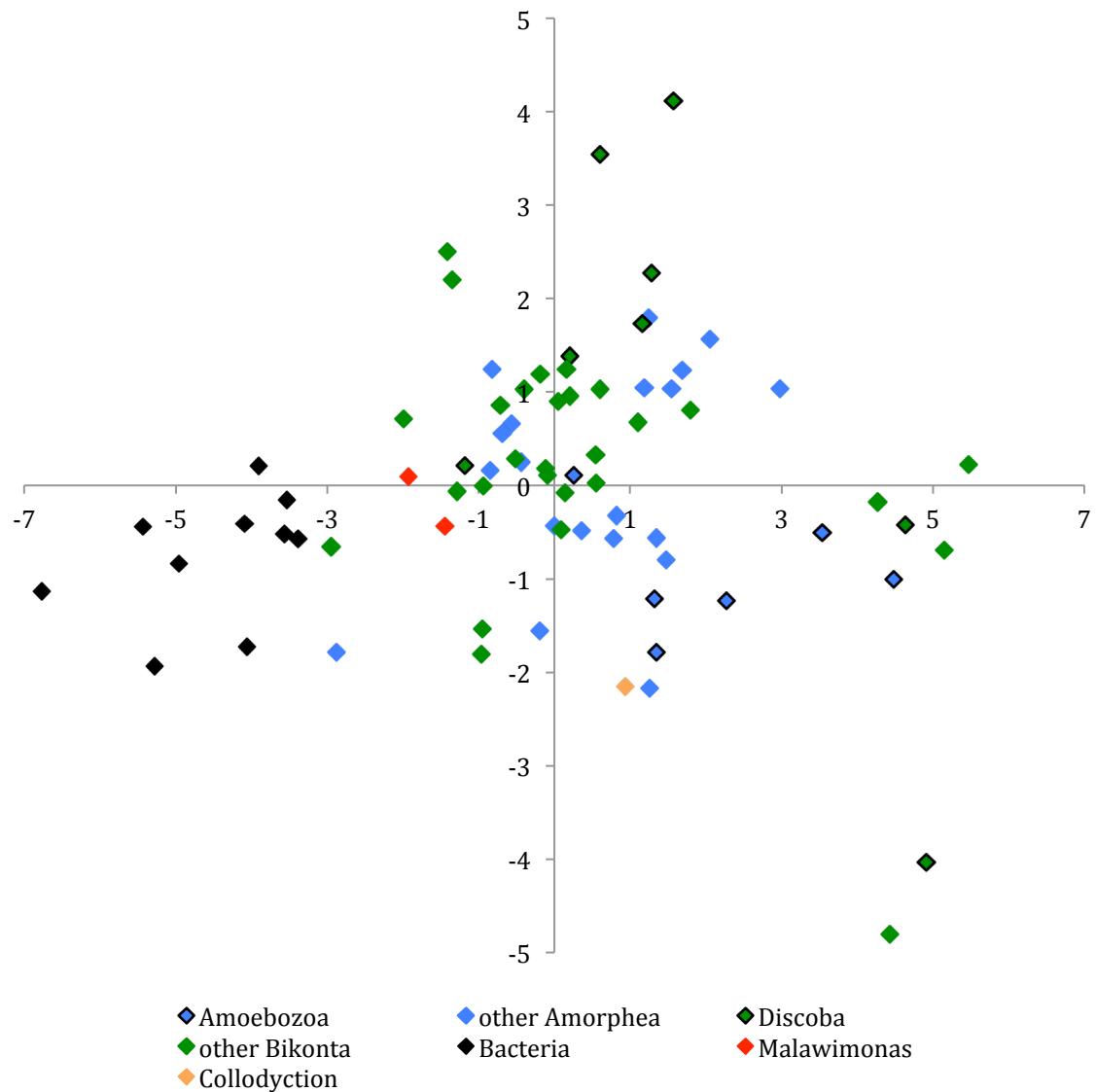
**Saturation plot of the EUBAC and He *et al.* 2014 datasets**

Saturation plots of the EUBAC dataset analyzed in this study (black dots and black regression line) and the EUBAC dataset analyzed in He *et al.* 2014 (blue dots and blue regression line) datasets. See Supplementary Material for method details.



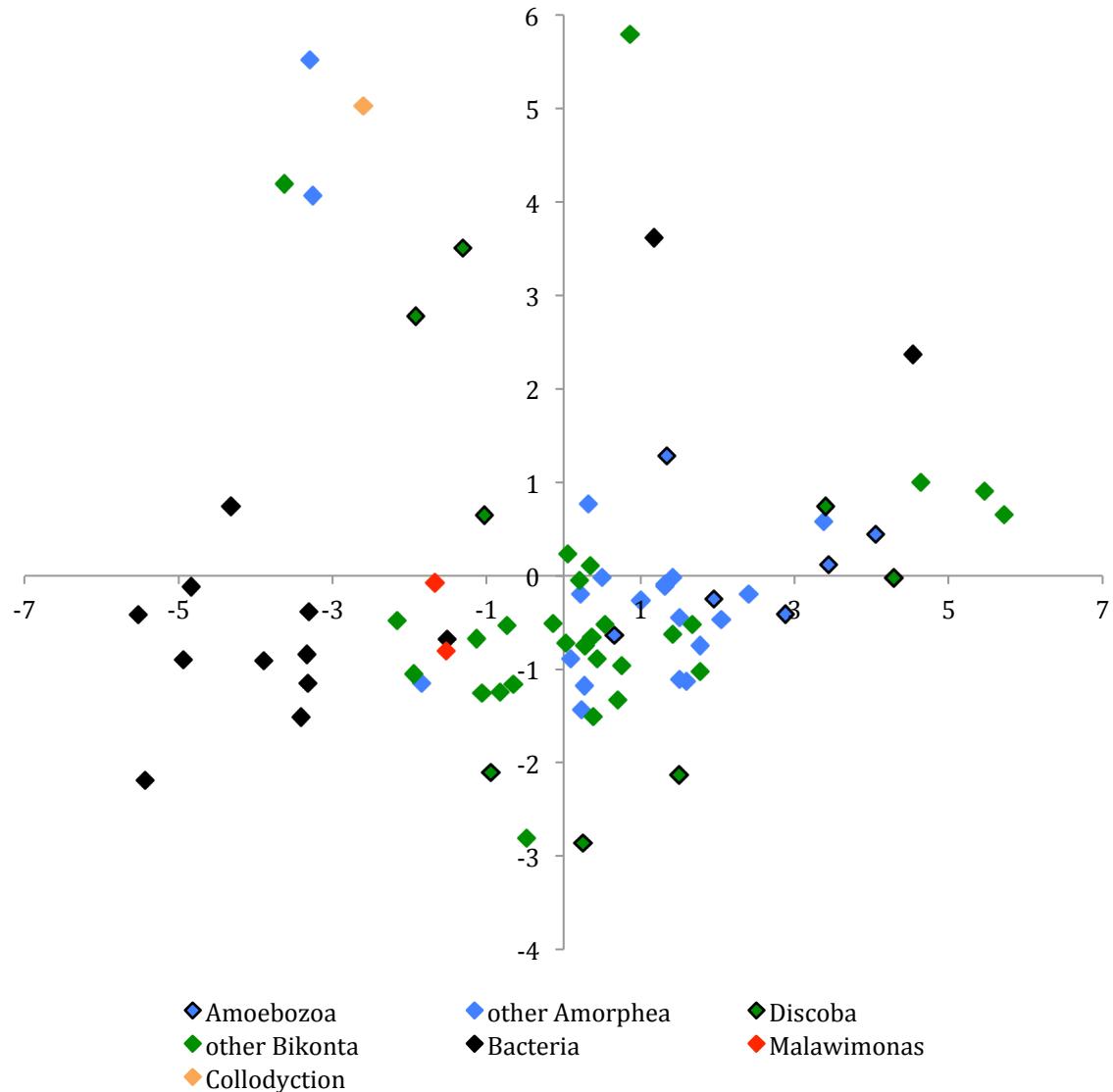
### **Principal component analysis of amino-acid frequencies of the ALPHA-PROT dataset**

The contribution of each axis are 32% and 11% for the X-axis and the Y-axis respectively.



### **Principal component analysis of amino-acid frequencies of the EUBAC dataset**

The contribution of each axis are 31% and 17% for the X-axis and the Y-axis respectively.



**Supplementary data S2:**

**outparalogs found in He *et al.* 2014  
dataset**

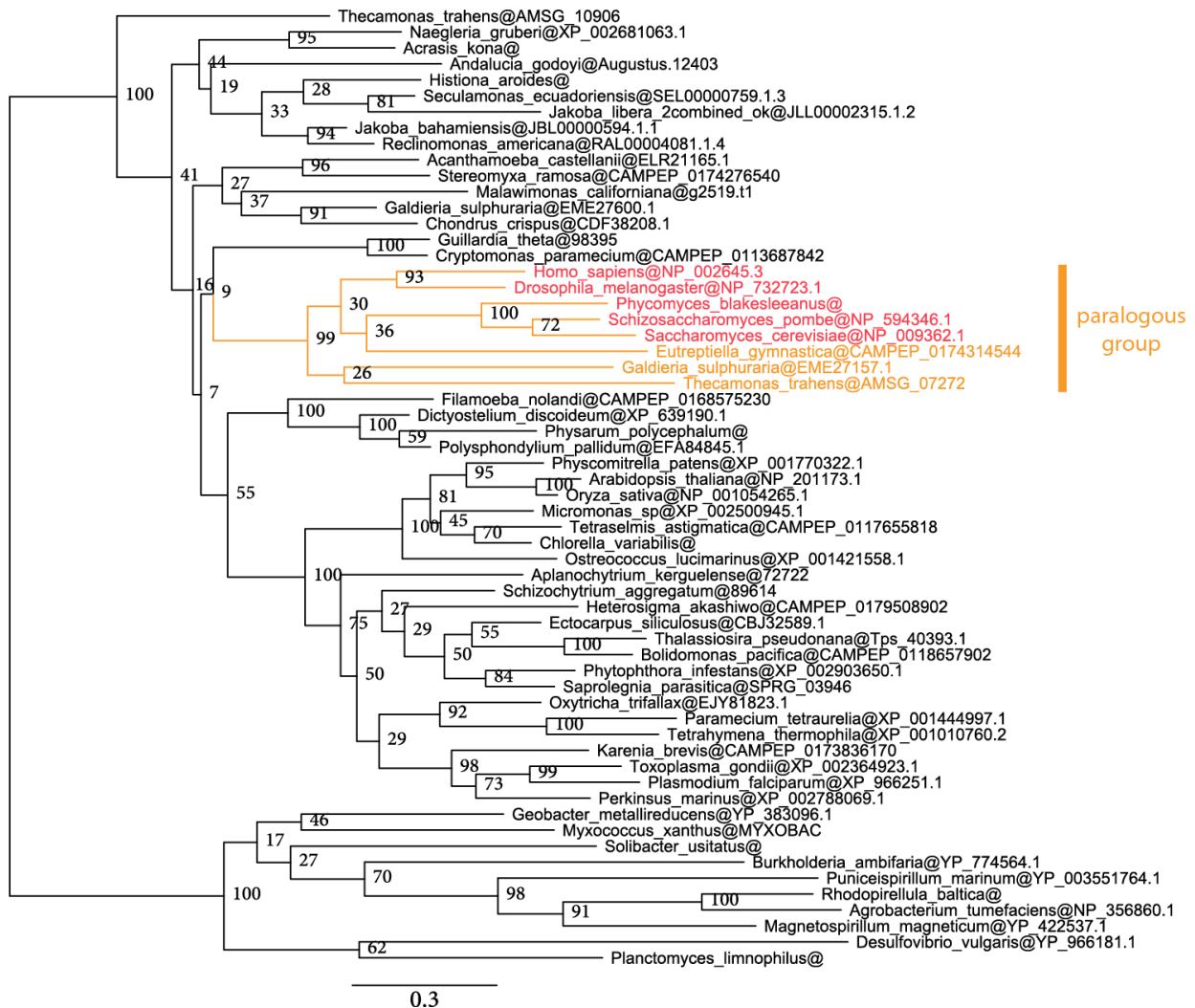
**List of outparalogs found in He *et al.* 2014 dataset**

marker	outparalogs
Protein 6	_ all opisthokonts
Protein 7	_ <i>Seculamonas ecuadoriensis</i> _ <i>Acanthamoeba</i>
Protein 9	_ <i>Seculamonas ecuadoriensis</i>
Protein 11	_ <i>Euglena gracilis</i>
Protein 14	_ <i>Jakoba bahamiensis</i> _ <i>Seculamonas ecuadoriensis</i>
Protein 24	_ <i>Naegleria gruberi</i> _ <i>Reclinomonas Americana</i> _ <i>Seculamonas ecuadoriensis</i> _ <i>Jakoba libera</i>

The following RAxML trees show, for each of these six phylogenetic markers, the eukaryotic orthologous group used by He *et al.* 2014.

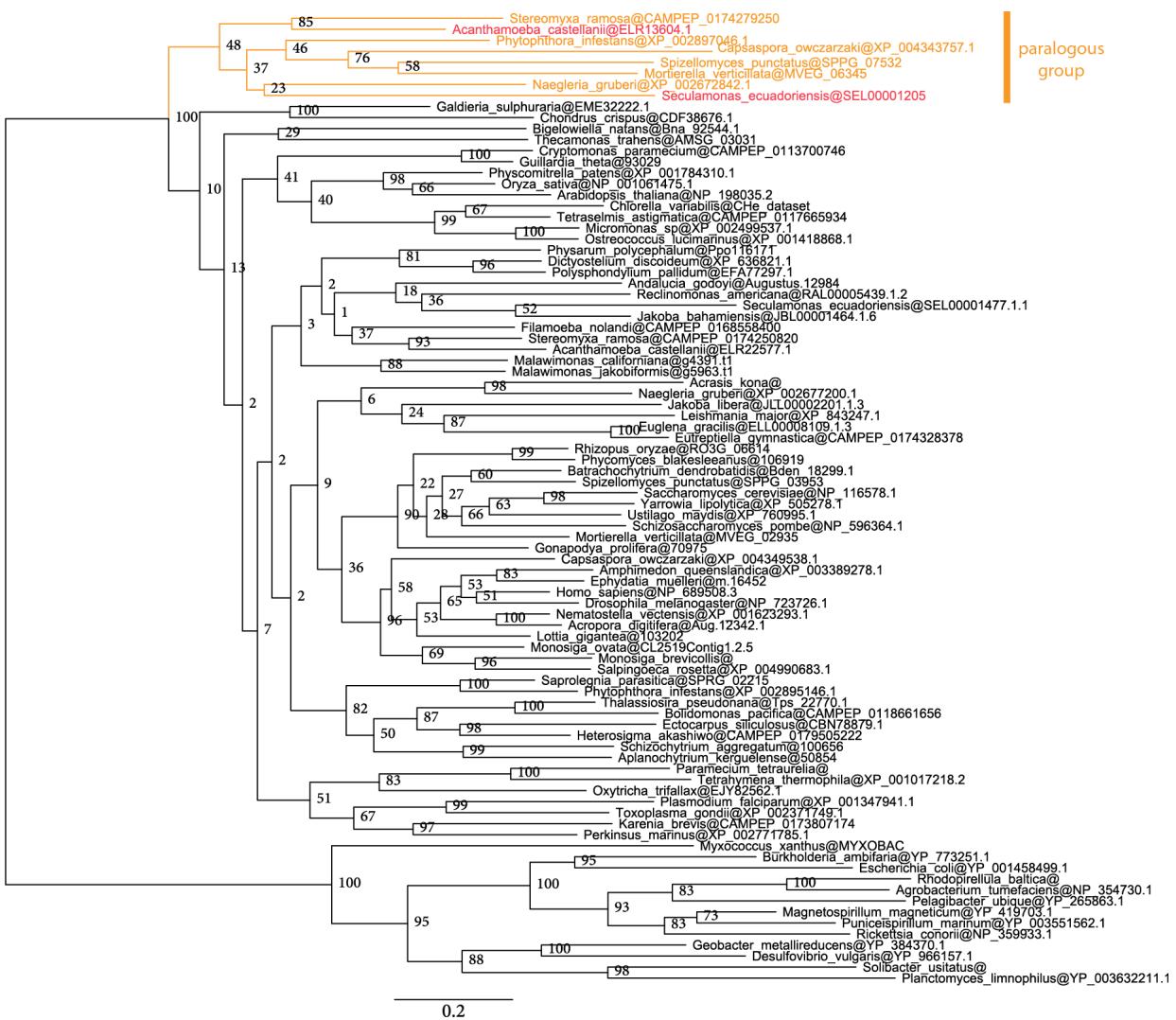
Paralogous group(s) are highlighted in orange and outparalogs detected in the He *et al.* 2014 dataset are highlighted in red.

The taxonomic sampling of paralogous groups has been reduced (we only kept few species representing most of eukaryotic supergroups). Alignments have been created and analyzed following the methods described in Materials and Methods.

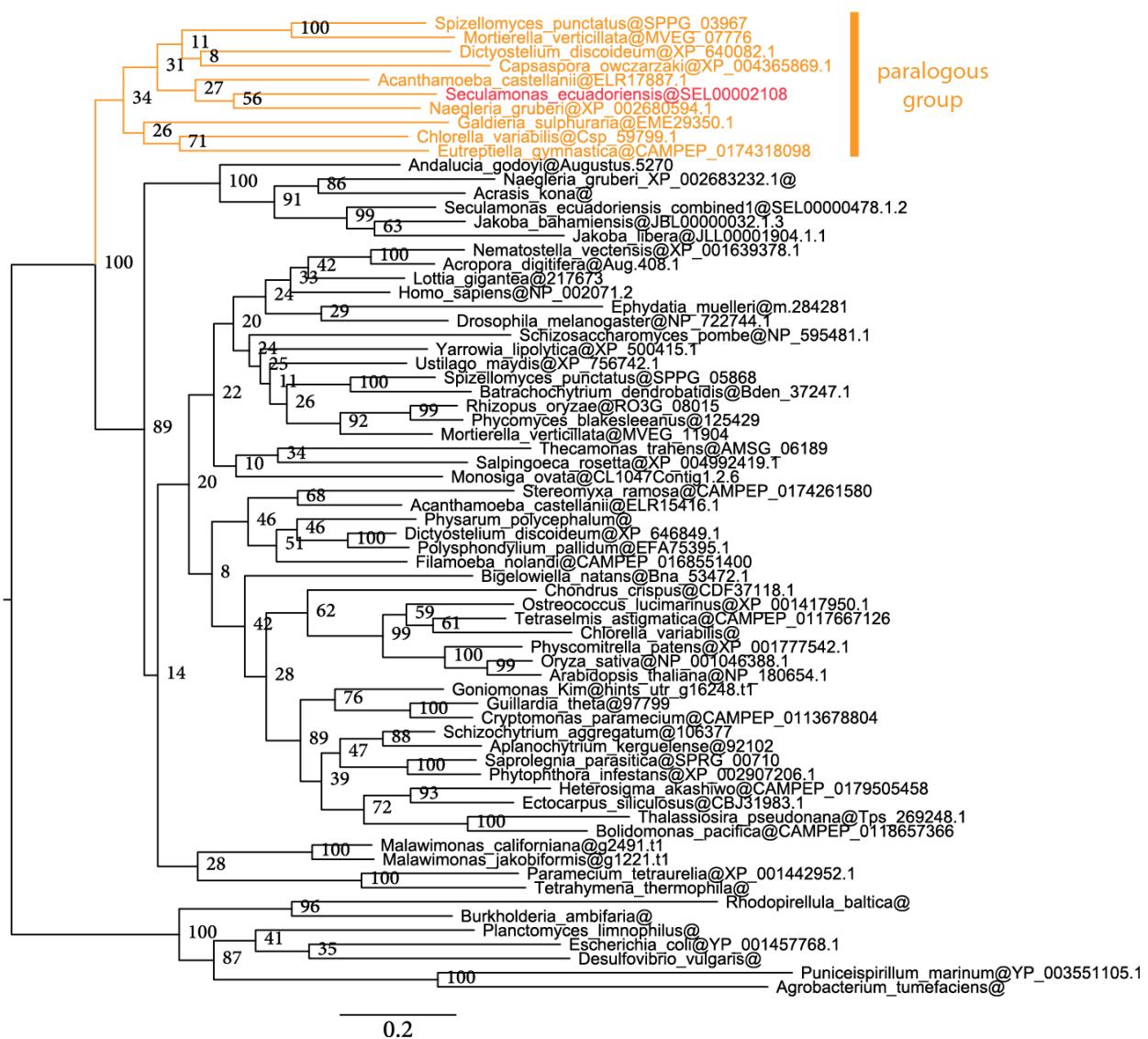


RAxML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 6** (325 positions).

Only a few representatives of opisthokonts have been kept in the alignment.

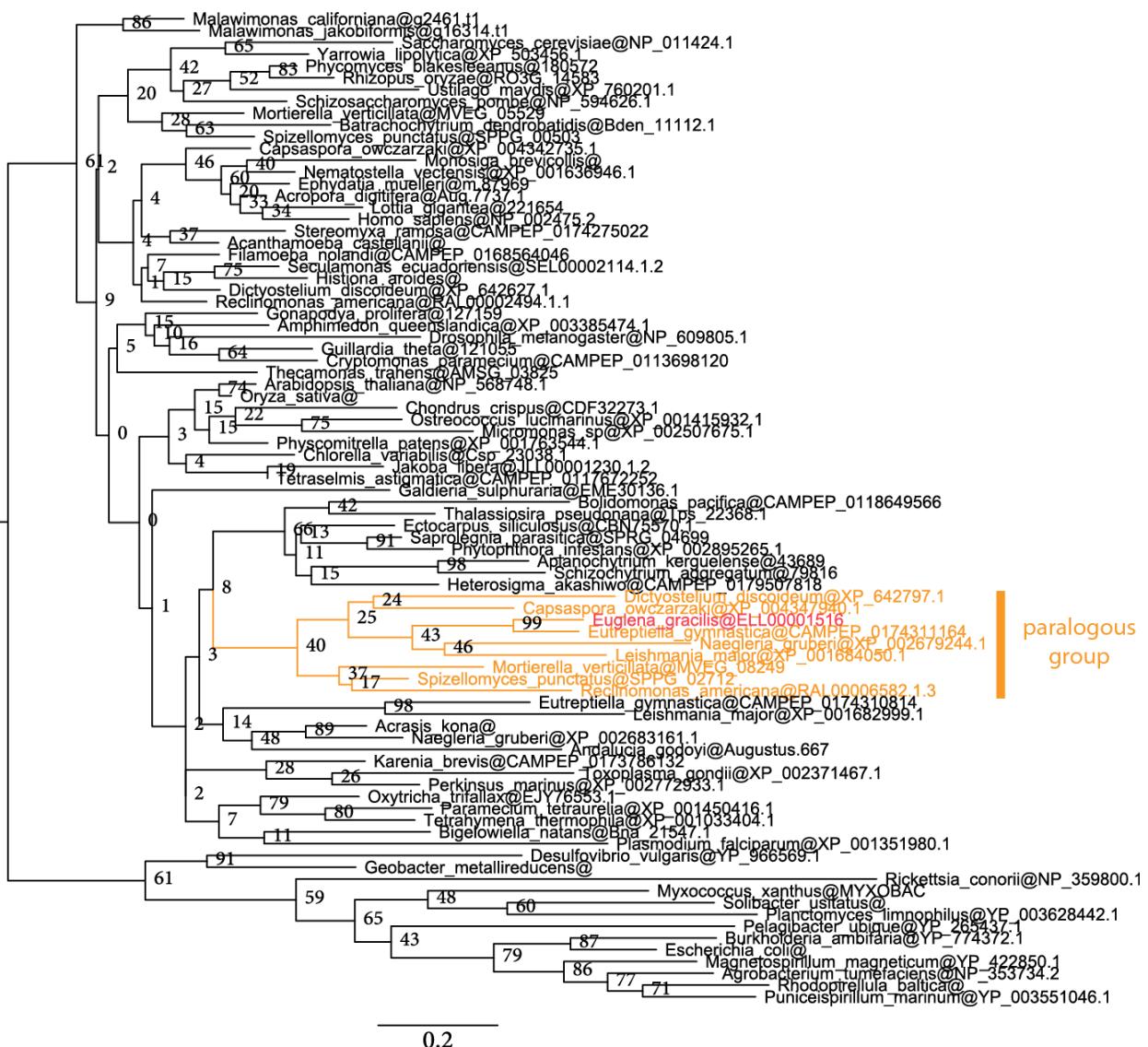


RAxML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 7** (520 positions).

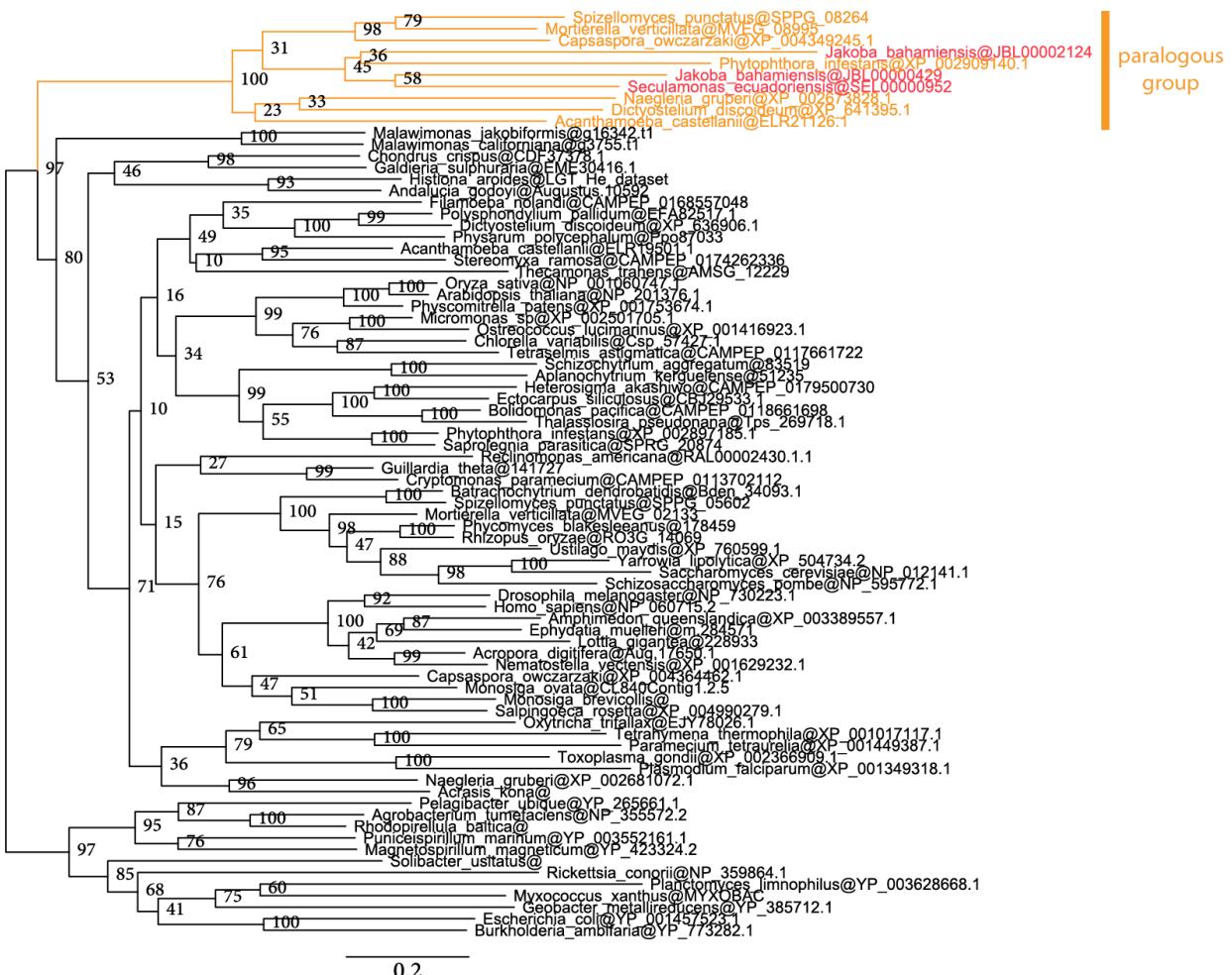


RAxML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 9** (357 positions).

The two *S. ecuadorensis* sequences (the ortholog SEL00000478 and the outparalog SEL00002108) have been combined into a chimeric sequence in He *et al.* 2014.

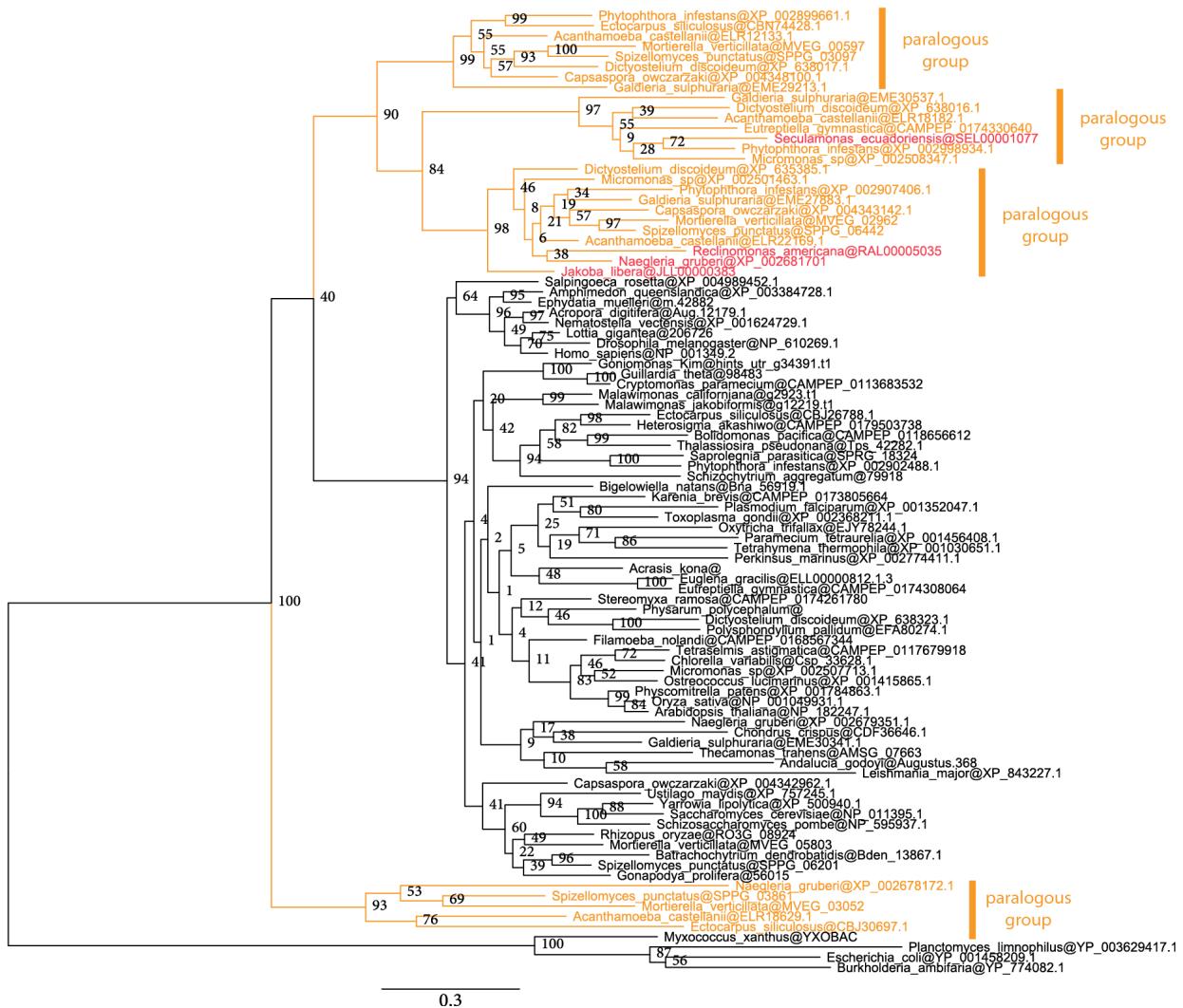


RAxML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 11** (176 positions).



RAXML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 14** (668 positions).

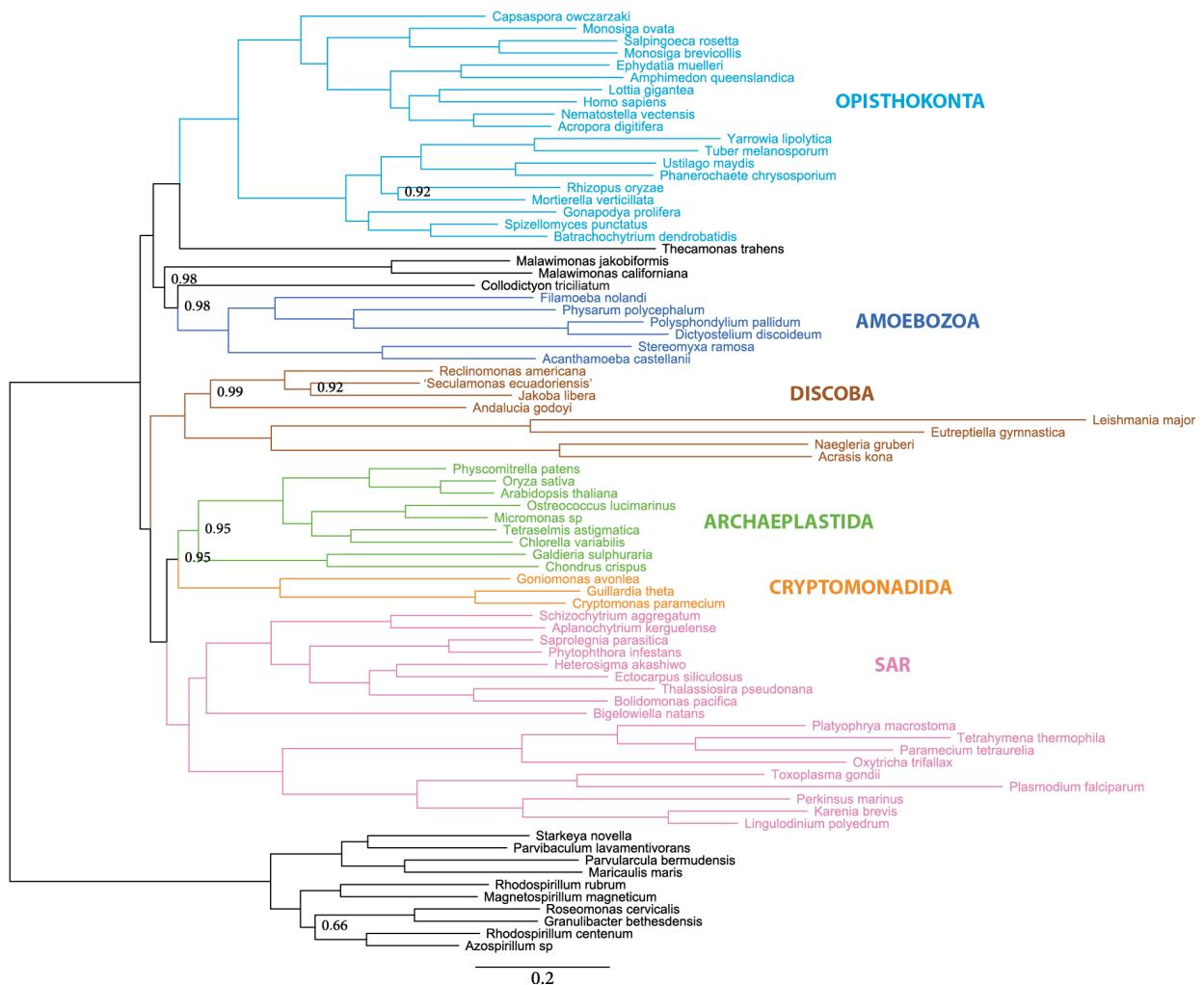
The two *J. bahamiensis* outparalogs have been combined into a chimeric sequence in He *et al.* 2014.



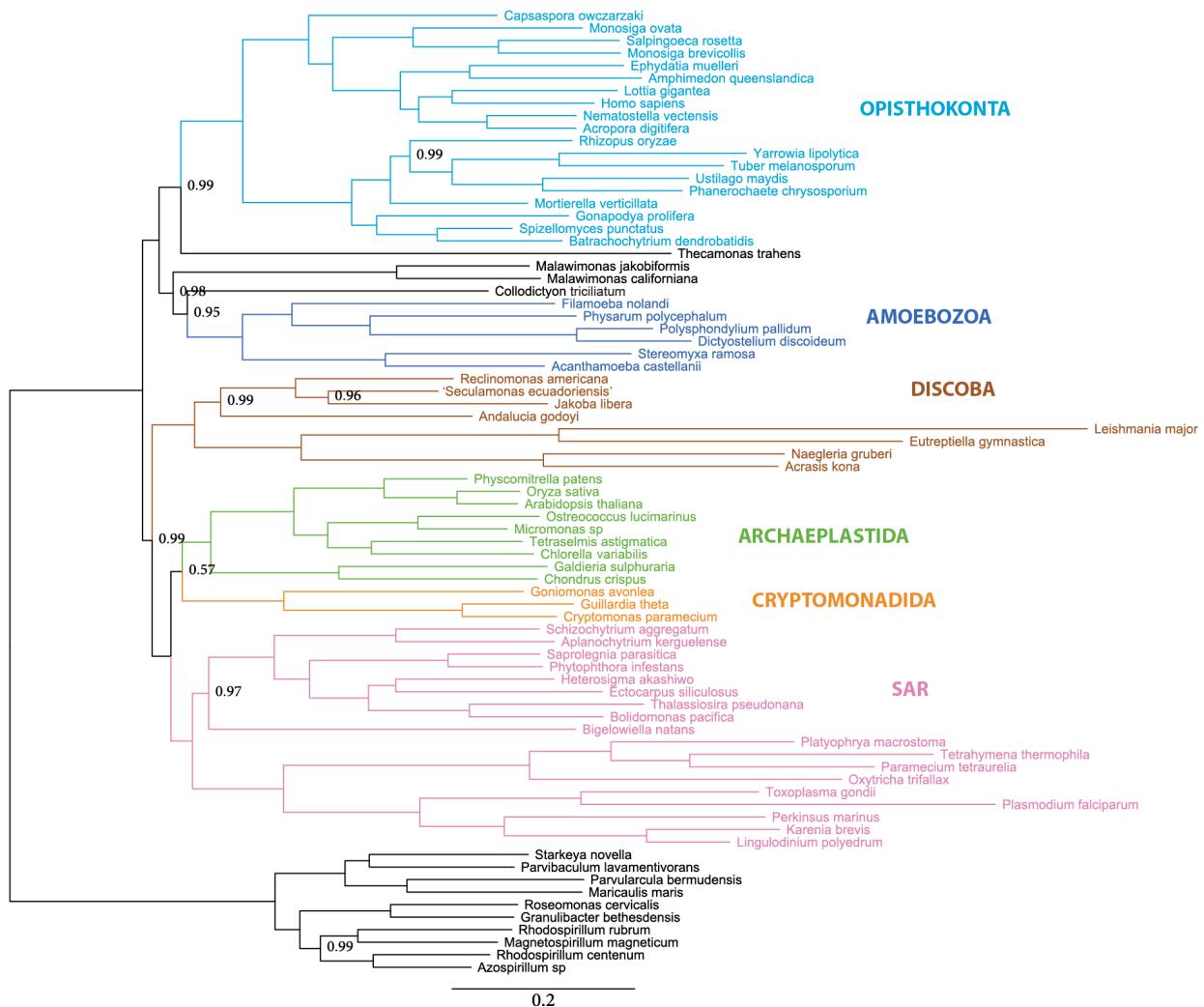
RAxML tree (LG model; 100 bootstrap replicates) obtained from the EUBAC marker **Protein 24** (554 positions).

## **Supplementary data S3:**

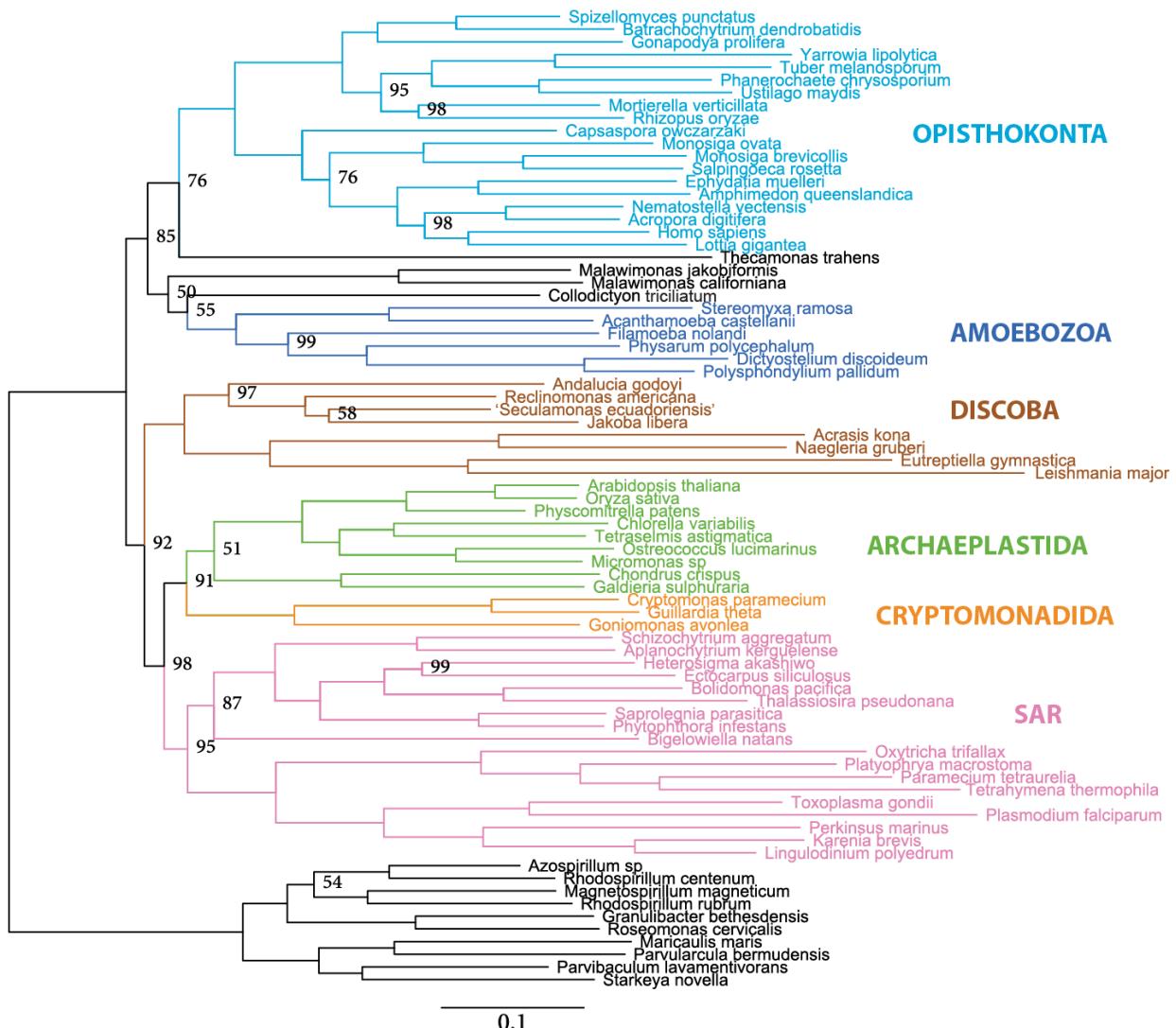
### **phylogenetic trees**



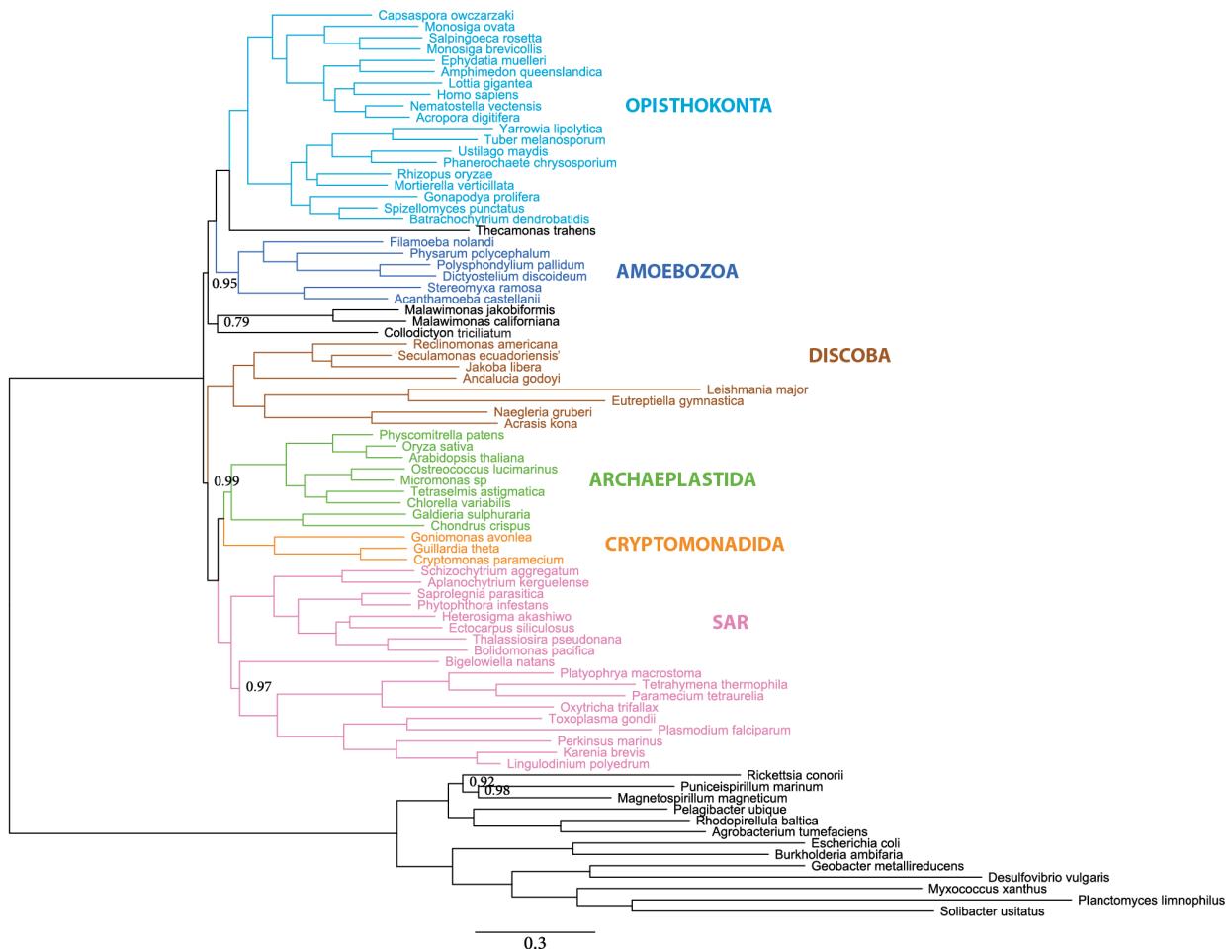
Bayesian consensus tree obtained from the **ALPHA-PROT** dataset under the **CAT-GTR +  $\Gamma_4$**  model. Branch support values equal to 1 are not displayed.



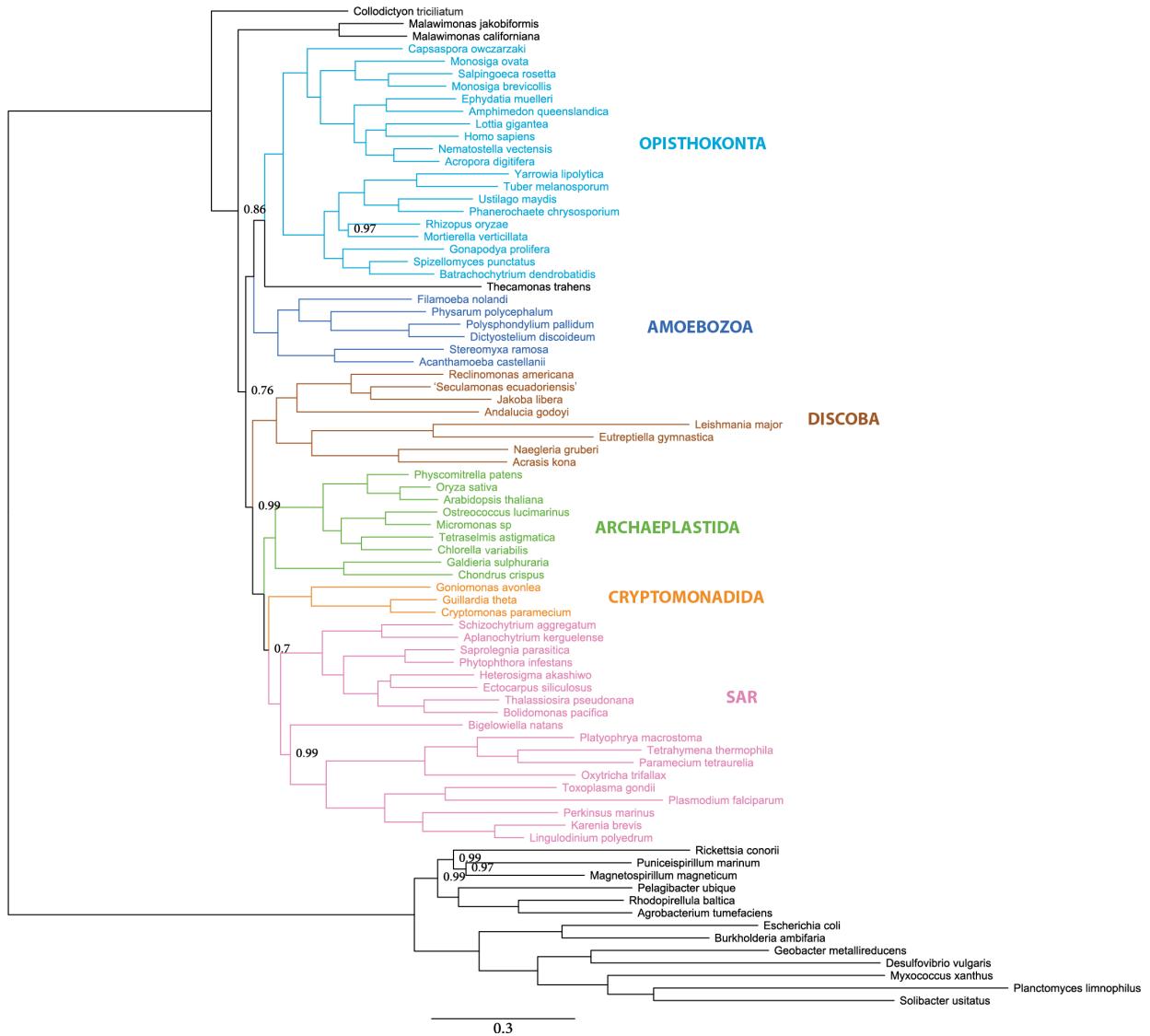
Bayesian consensus tree obtained from the **ALPHA-PROT** dataset under the **CAT +  $\Gamma_4$**  model. Branch support values equal to 1 are not displayed.



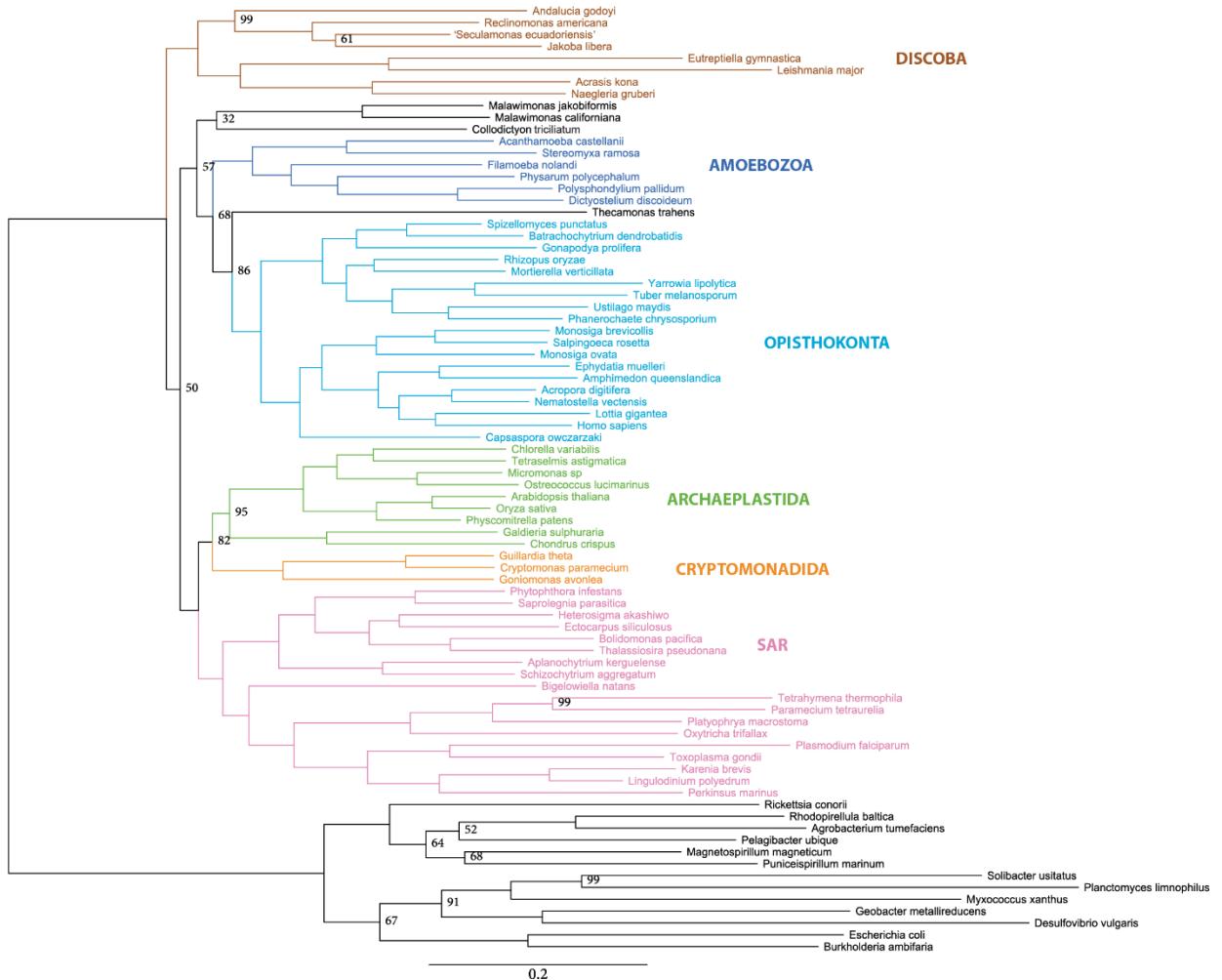
Best RAXML tree obtained from the **ALPHA-PROT** dataset under the **GTR + Γ4** model. Branch support values equal to 100 are not displayed.



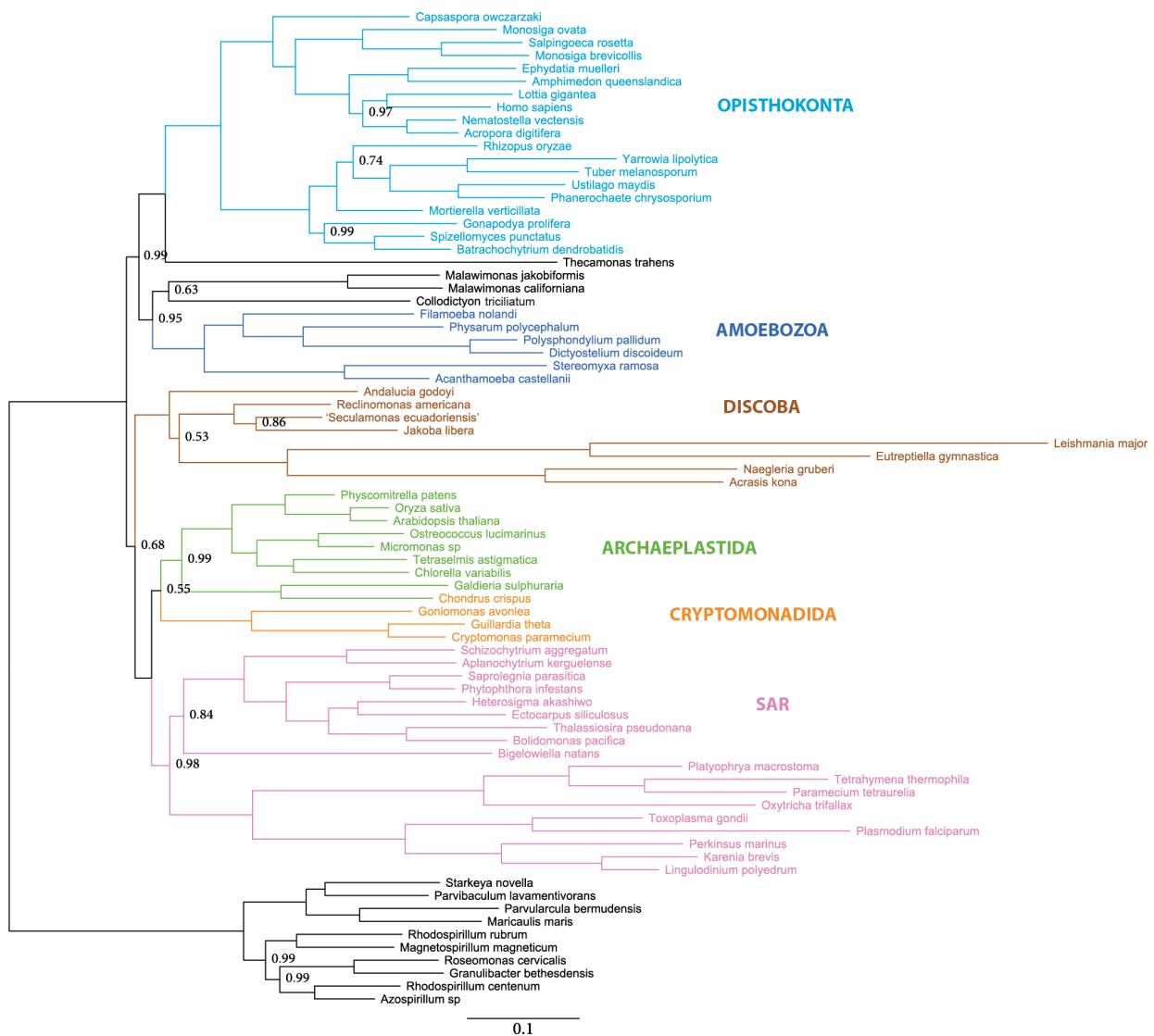
Bayesian consensus tree obtained from the **EUBAC** dataset under the **CAT-GTR + Γ4** model. Branch support values equal to 1 are not displayed.



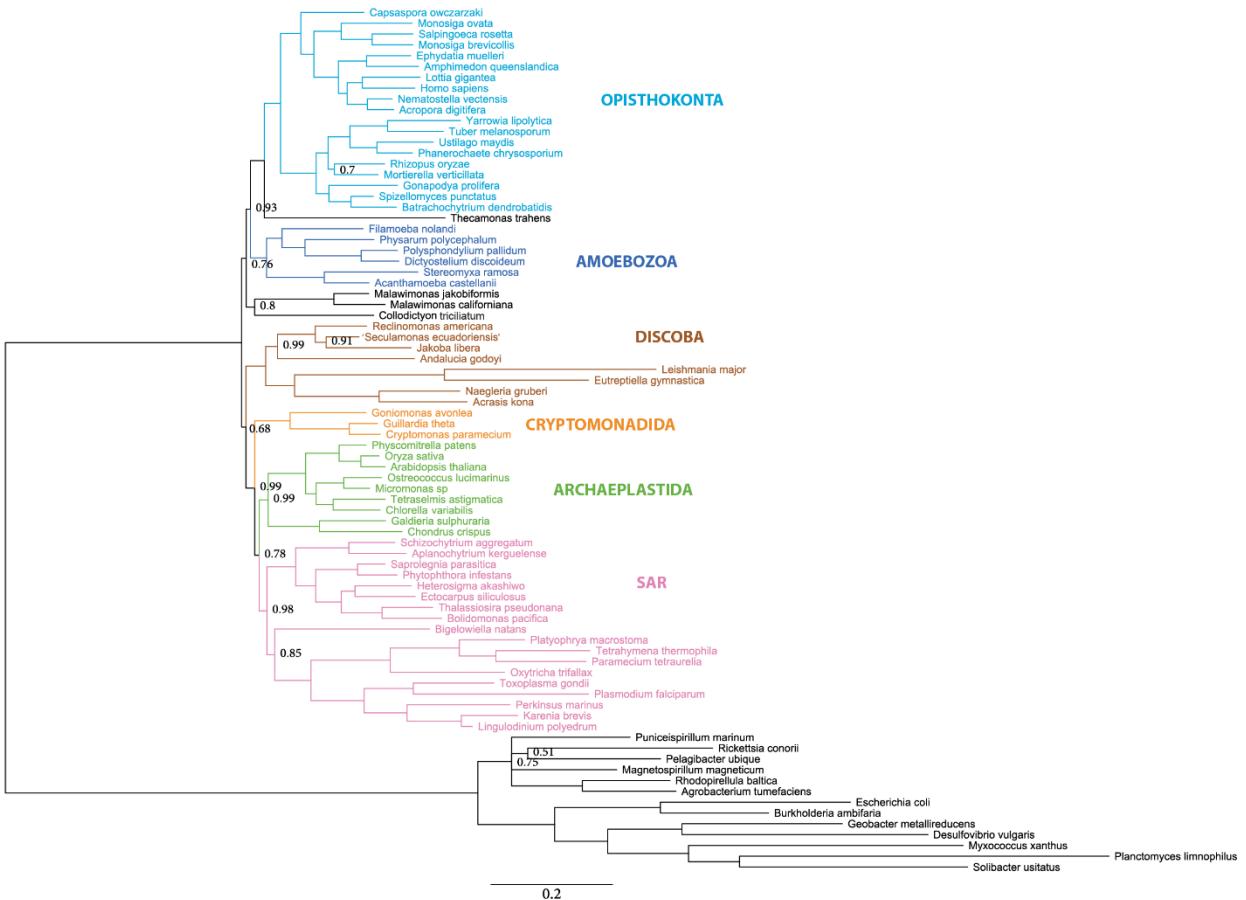
Bayesian consensus tree obtained from the **EUBAC** dataset under the **CAT + Γ4** model. Branch support values equal to 1 are not displayed.



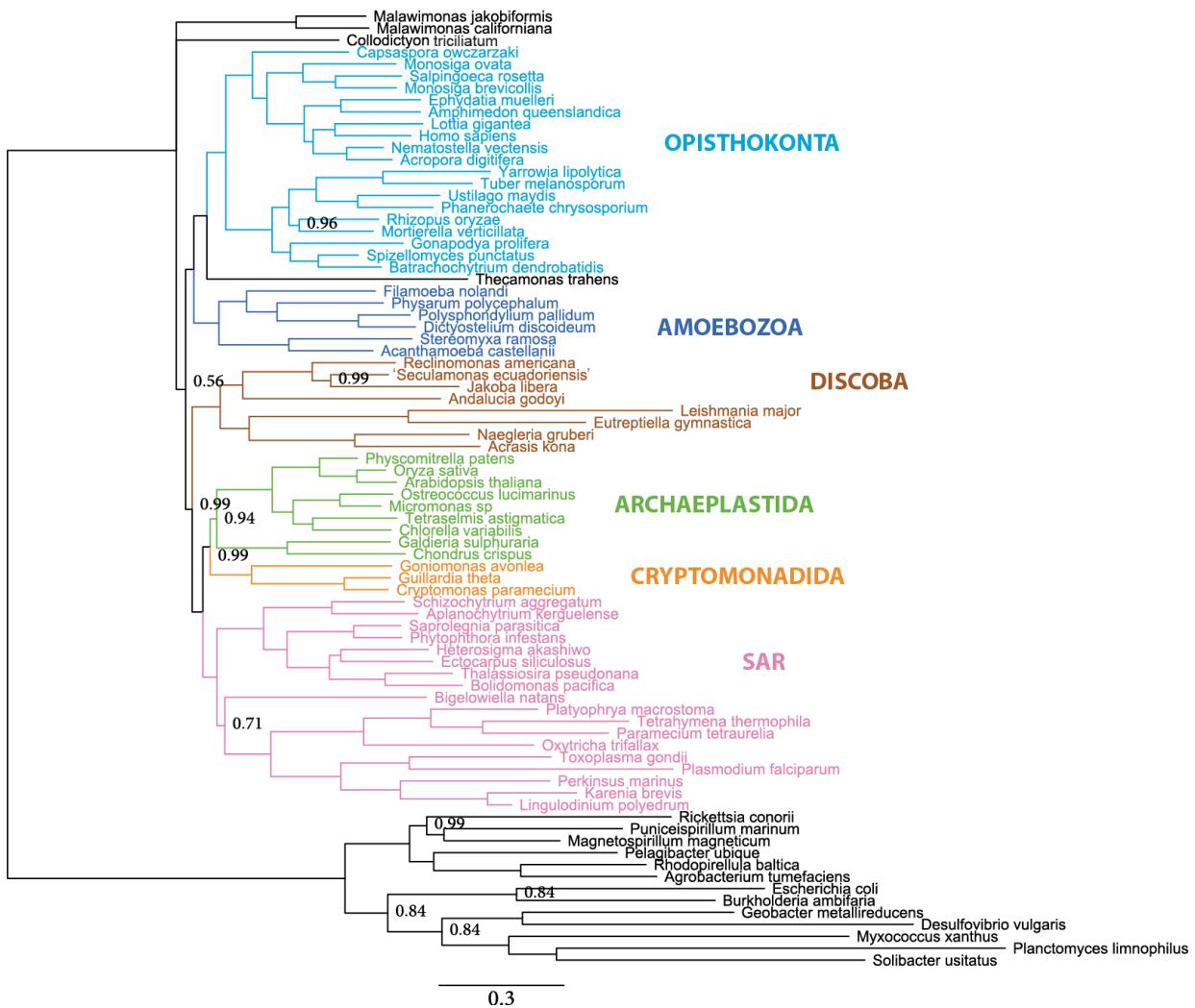
Best RAXML tree obtained from the **EUBAC** dataset under the **GTR + Γ4** model.  
Branch support values equal to 100 are not displayed.



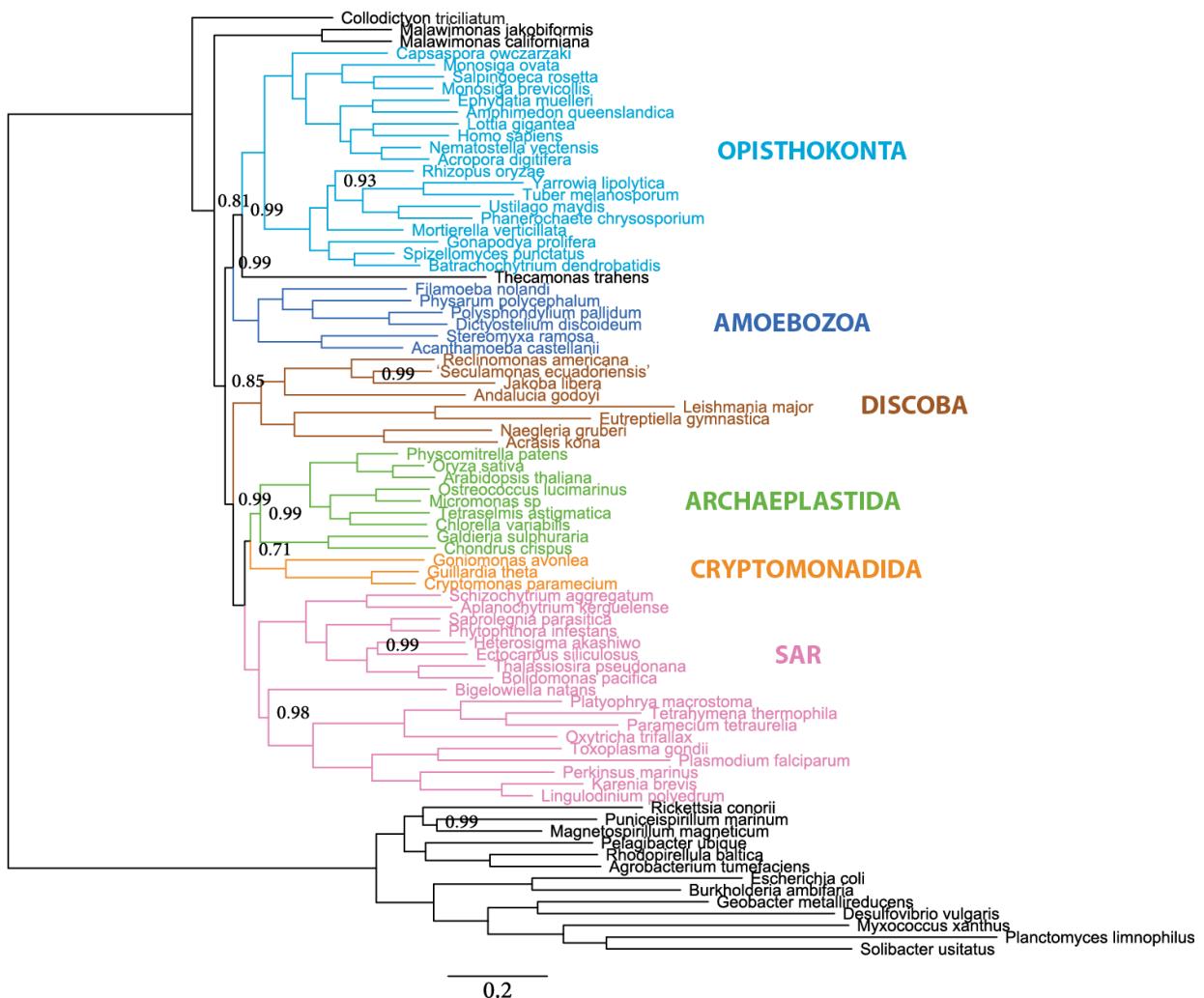
Bayesian consensus tree obtained from the **ALPHA-PROT** dataset recoded by the **dayhoff6 recoding scheme** under the **CAT-GTR + Γ4** model. Branch support values equal to 1 are not displayed.



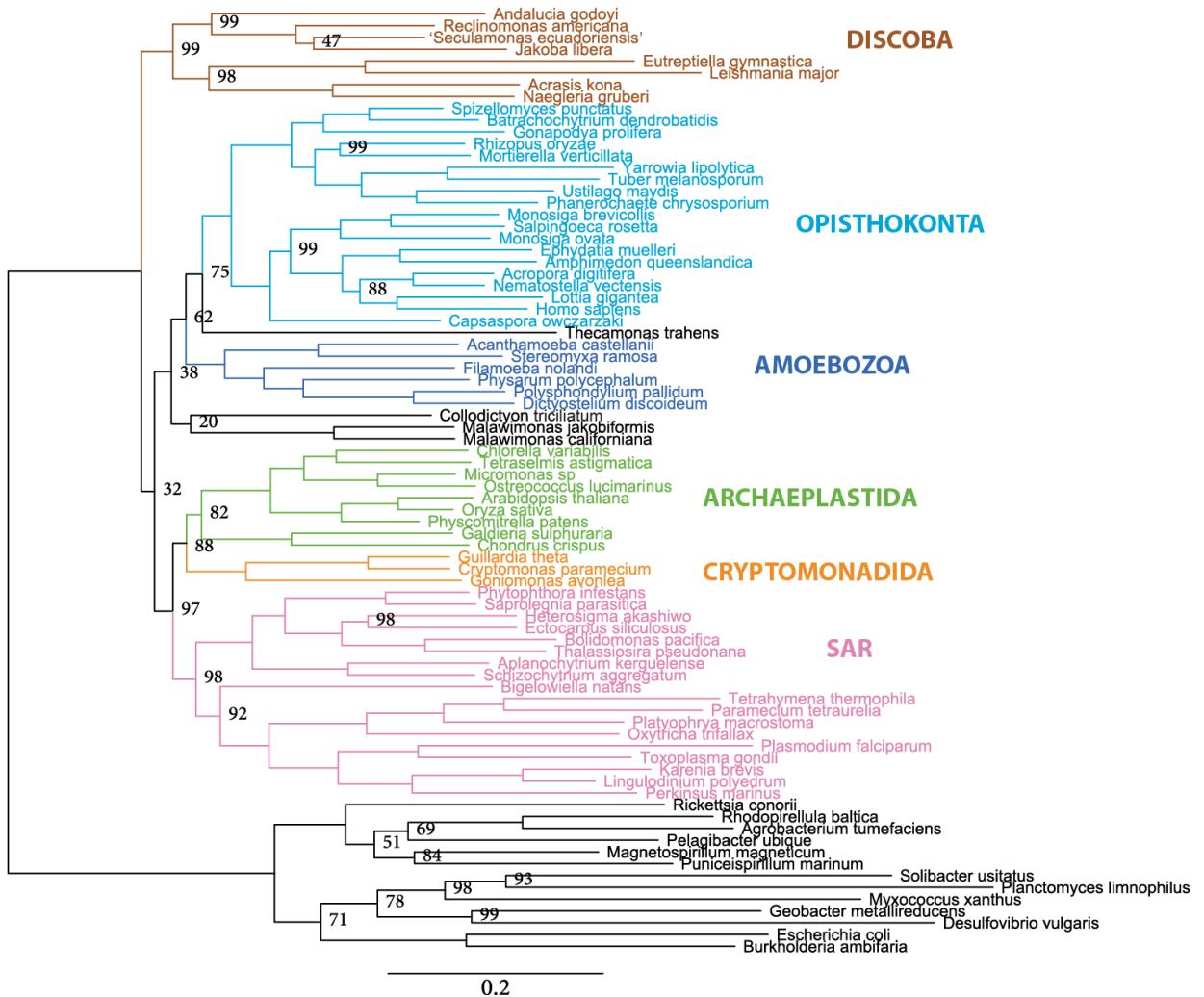
Bayesian consensus tree obtained from the **EUBAC** dataset recoded by the **dayhoff6 recoding scheme** under the **CAT-GTR + Γ4** model. Branch support values equal to 1 are not displayed.



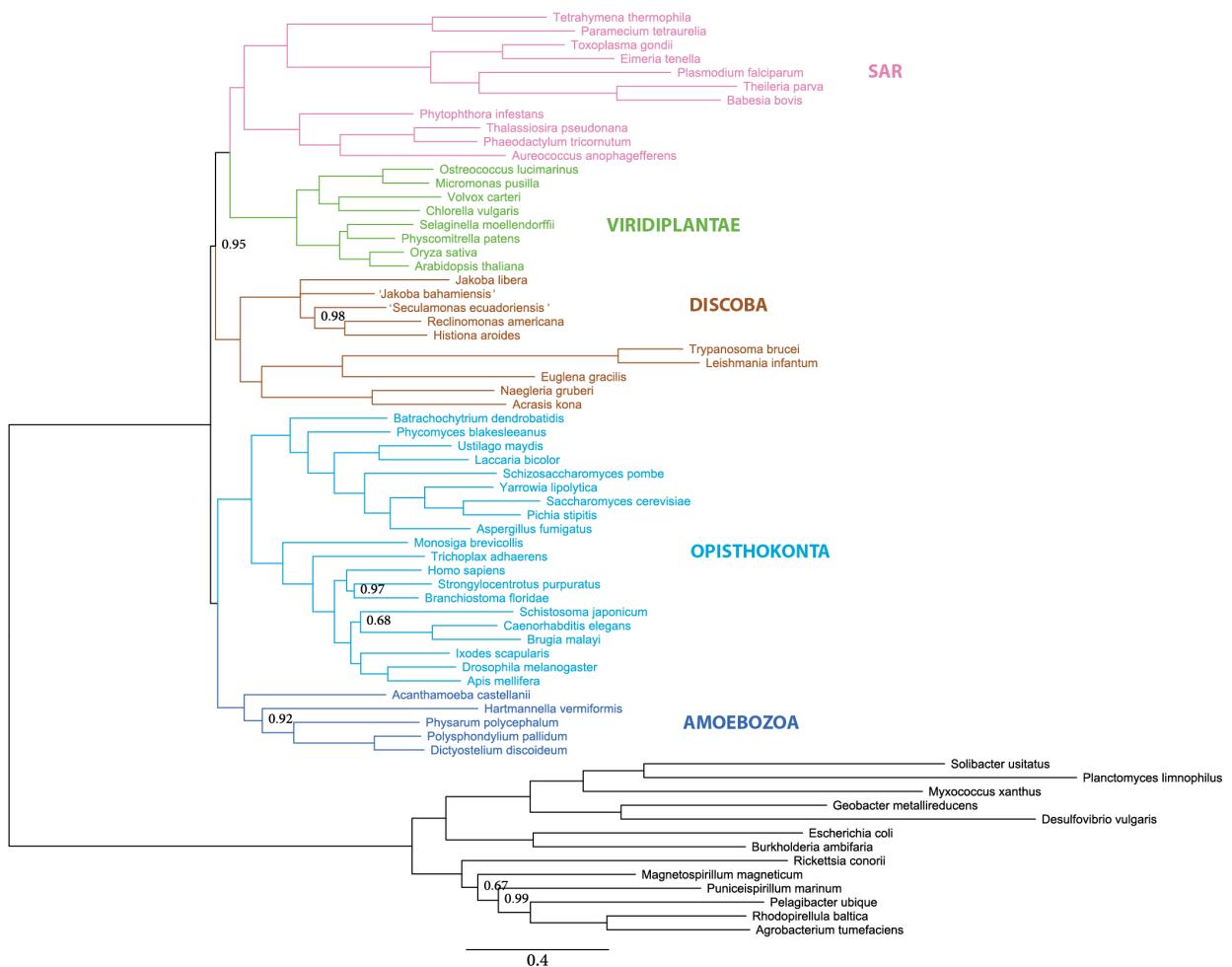
Bayesian consensus tree obtained from the **EUBAC reduced** dataset (i.e. minus the 10 most divergent markers) under the **CAT-GTR + Γ4** model. Branch support values equal to 1 are not displayed.



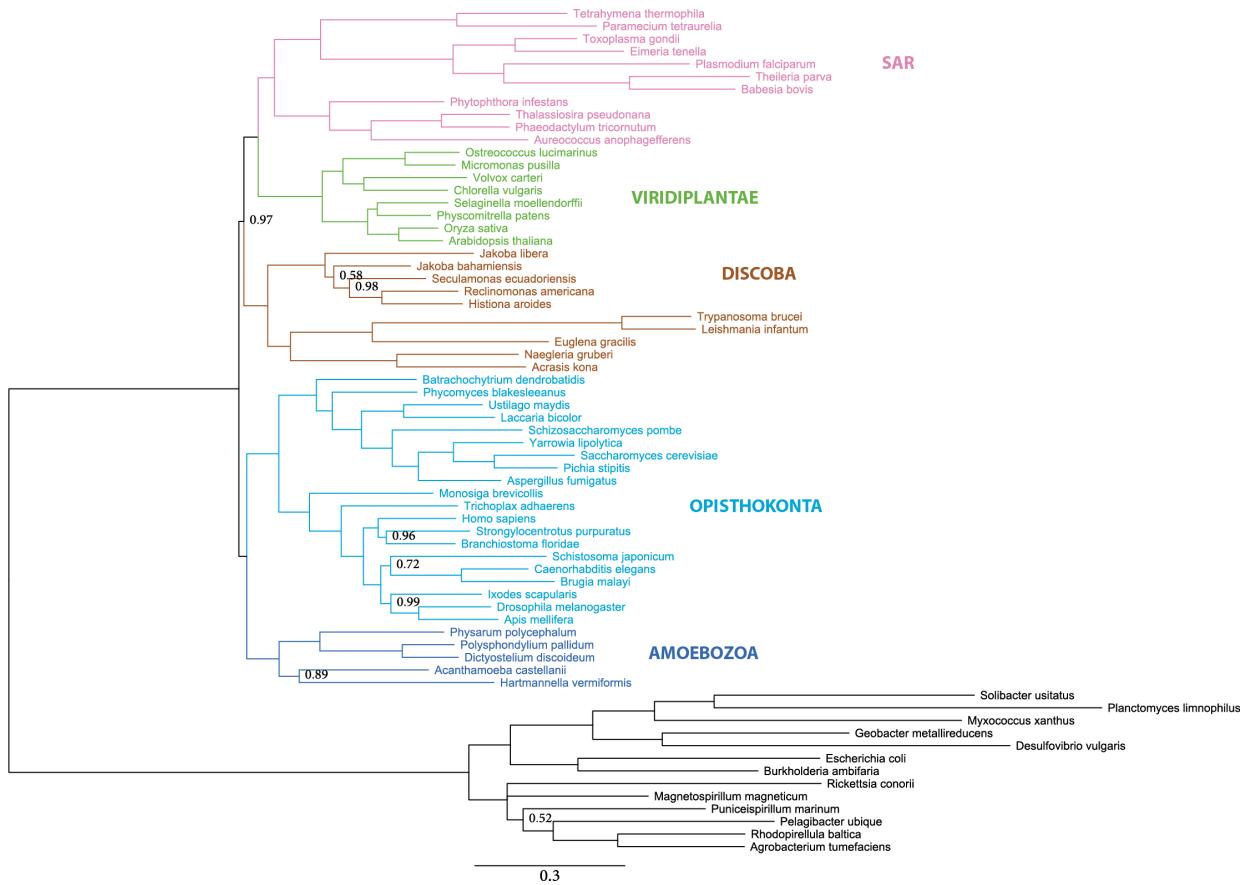
Bayesian consensus tree obtained from the **EUBAC reduced** dataset (i.e. minus the 10 most divergent markers) under the **CAT + Γ4** model. Branch support values equal to 1 are not displayed.



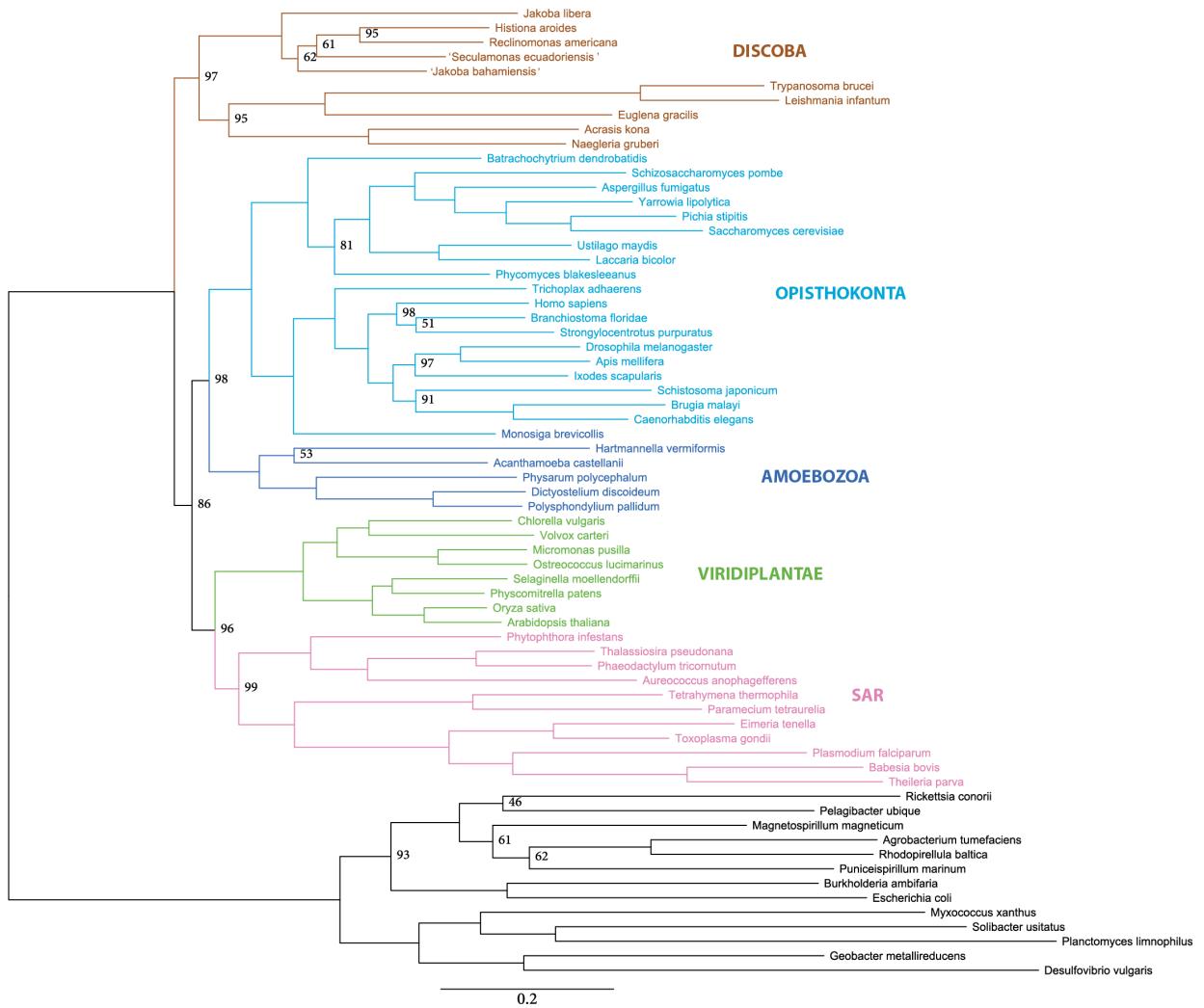
Best RAXML tree obtained from the **EUBAC reduced** dataset (i.e. minus the 10 most divergent markers) under the **GTR + Γ4** model. Branch support values equal to 100 are not displayed.



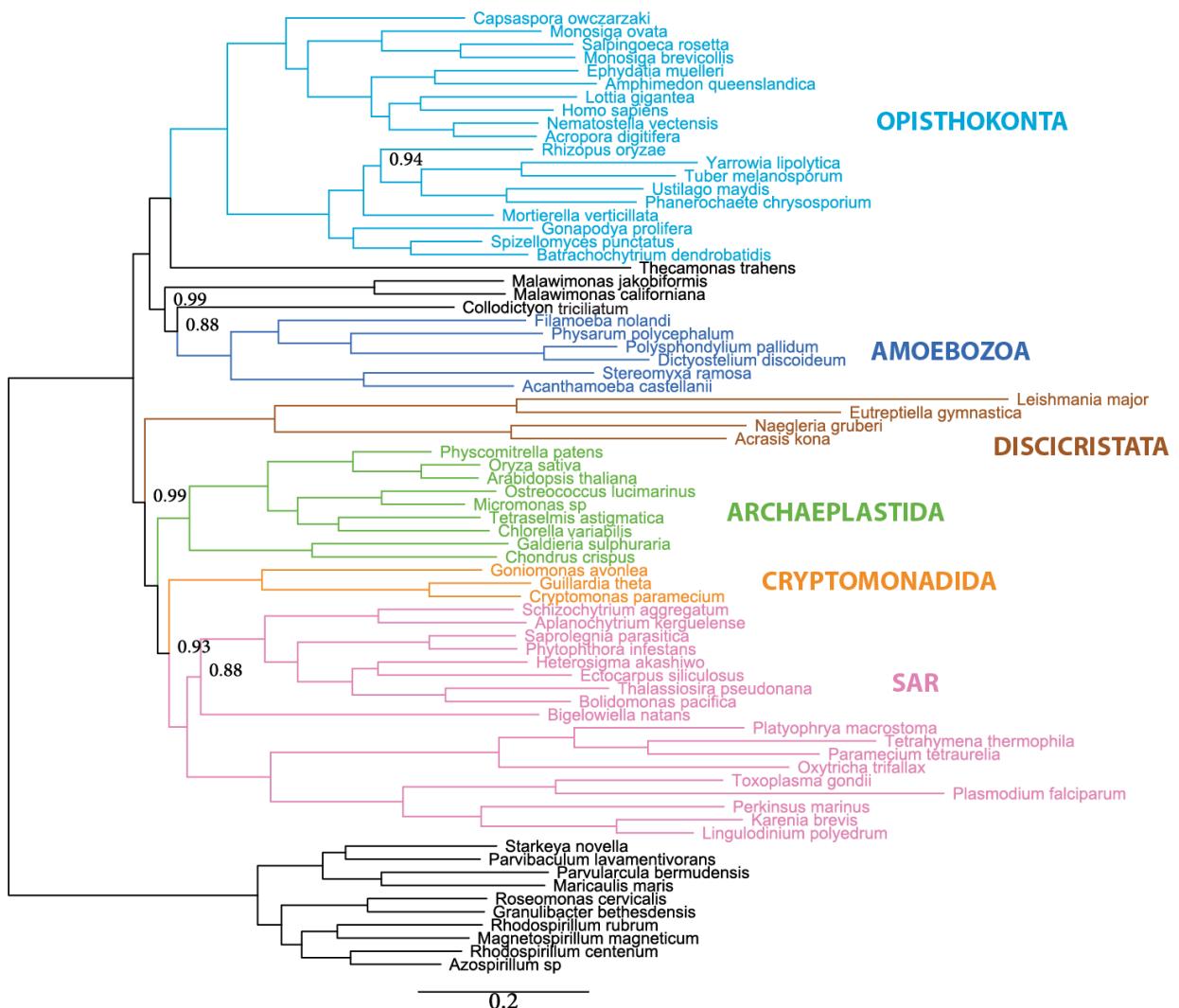
Bayesian consensus tree obtained from the **EUBAC He et al. 2014** dataset with distant paralogs replaced by "X" under the **CAT-GTR + Γ4** model. Branch support values equal to 1 are not displayed.



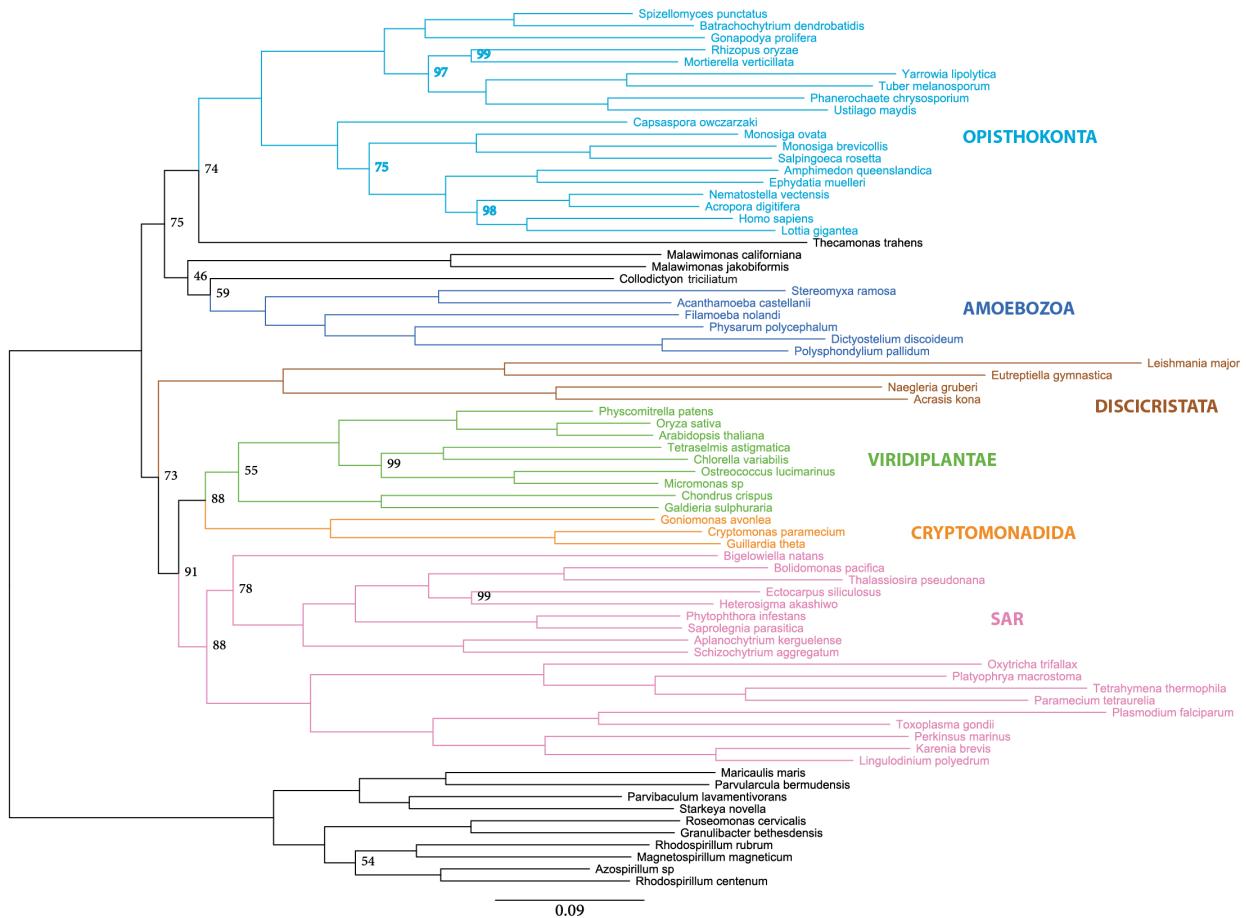
Bayesian consensus tree obtained from the **EUBAC He et al. 2014** dataset with distant paralogs replaced by “X” under the **CAT + Γ4** model. Branch support values equal to 1 are not displayed.



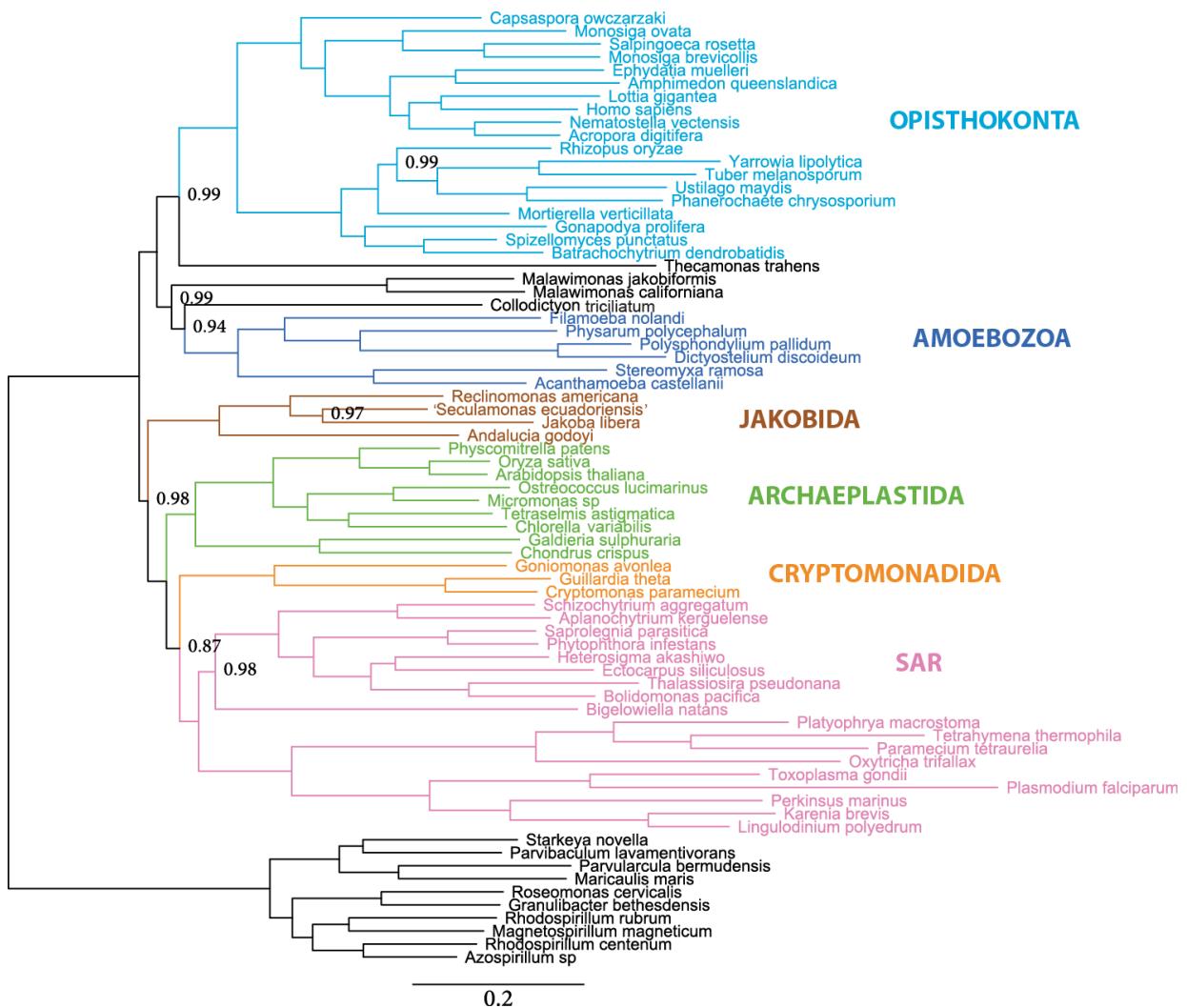
Best RAXML tree obtained from the **EUBAC He et al. 2014** dataset with distant paralogs replaced by “X” under the **GTR + Γ4** model. Branch support values equal to 100 are not displayed.



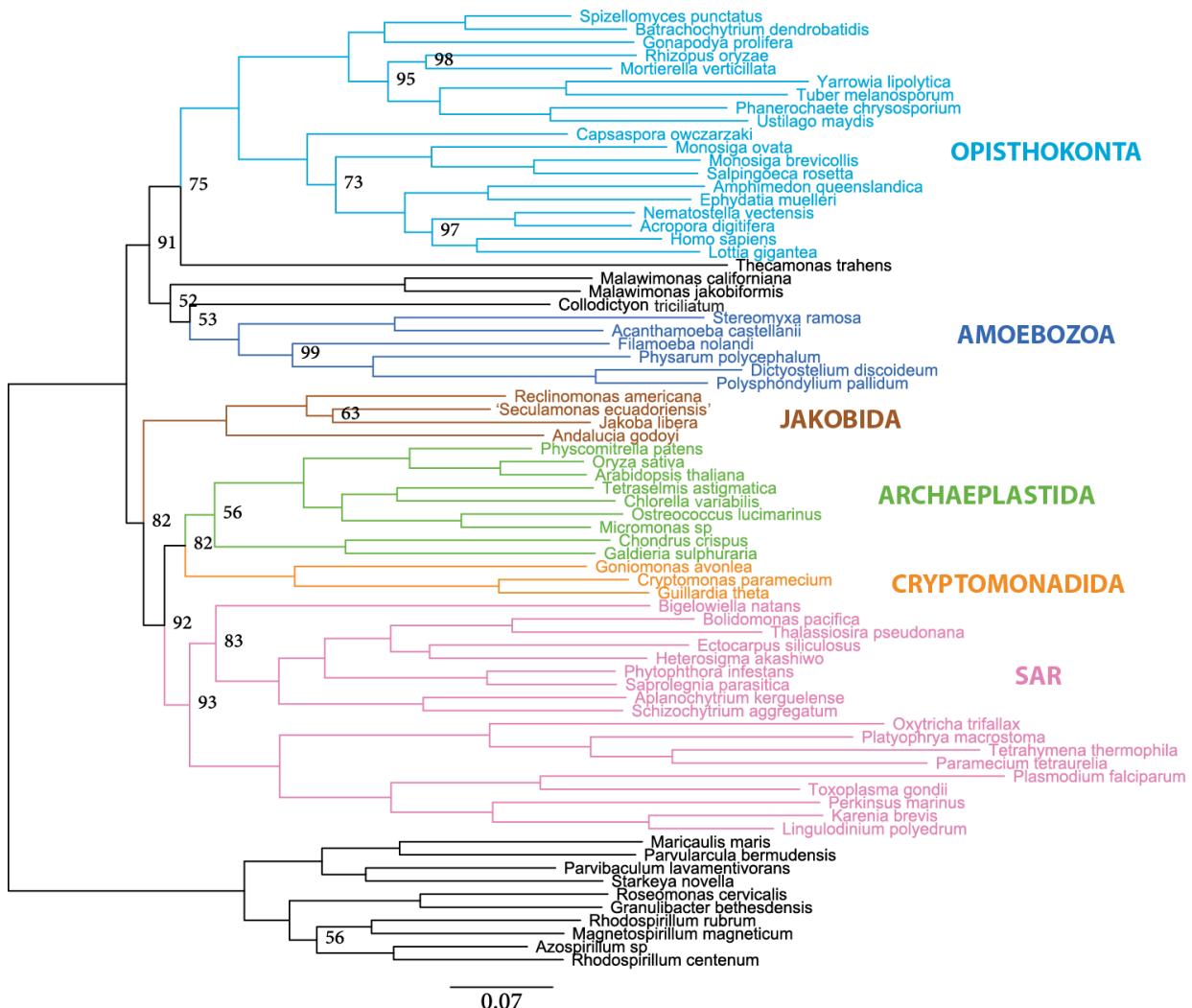
Bayesian consensus tree obtained from the **ALPHA-PROT** dataset **without Jakobida** under the **CAT + Γ4** model. Branch support values equal to 1 are not displayed.



Best RAXML tree obtained from the **ALPHA-PROT** dataset **without Jakobida** under the **GTR + Γ4** model. Branch support values equal to 100 are not displayed.



Bayesian consensus tree obtained from the **ALPHA-PROT** dataset **without** **Discicristata** under the **CAT + Γ4** model. Branch support values equal to 1 are not displayed.



Best RAXML tree obtained from the **ALPHA-PROT** dataset **without Discicristata** under the **GTR + Γ4** model. Branch support values equal to 100 are not displayed.

## **Supplementary data S4:**

### **single-gene congruence tests**

Congruence tests using Conclusador applied on the datasets built in Derelle and Lang 2012 and He *et al.* 2014 reveal an absence of incongruence of phylogenetic signal.

In contrast, Conclusador clusters the ALPHA-PROT and EUBAC datasets in two clusters of genes. However, two independent observations demonstrate that this incongruence of phylogenetic signal is quantitative (i.e. different amounts of phylogenetic signal) rather than qualitative (i.e. conflicting phylogenetic signals), a common artefact affecting algorithms aiming to compare the phylogenetic signal between genes (Leigh *et al* 2011; Leigh *et al* 2011):

\_ we estimated the distribution of alignment sizes, approximated by the number of characters contained in the alignments (i.e. the product of the number of positions by the number of species), among clusters. This analysis reveals that, concerning both the ALPHA-PROT and the EUBAC datasets, the cluster0 is composed of large single-gene matrices while the cluster1 is composed of small single-gene matrices. The alignment sizes are significantly different between the two clusters as indicated by the two-tailed Mann-Whitney U-Test. In other words, Conclusador has clustered the genes into two groups according the size of their matrix.

\_ we then have independently performed ML GTR and CAT analyses (same parameters as those described in Material and Methods) of cluster0 and cluster1 of the ALPHA-PROT dataset to test the presence of conflicting phylogenetic signals between the two cluster of genes. As expected, the large cluster0, representing two thirds of the ALPHA-PROT dataset, gives a very similar topology as the one shown in Figure 1 (the only exception are the deep Opimoda relationships, which are unsupported here) while the small cluster1, representing only one third of the ALPHA-PROT dataset, recovers all consensual eukaryotic supergroups but fails to infer their deep relationships.

Importantly enough, (i) we can not observe any supported difference between the cluster0 and cluster1 topologies, indicating that there is no conflicting phylogenetic signal between the two clusters, and (ii) the Discoba group is found monophyletic in all four phylogenetic trees, indicating that Conclusador did not detect any conflicting phylogenetic signal between these species.

Leigh JW, Schliep K, Lopez P, Bapteste E. 2011. Let them fall where they may: congruence analysis in massive phylogenetically messy data sets. Mol Biol Evol. 2011 Oct;28(10):2773-85.

Leigh JW, Lapointe FJ, Lopez P, Bapteste E. 2011. Evaluating phylogenetic congruence in the post-genomic era. Genome Biol Evol. 2011;3:571-87.

### **Summary of congruence tests performed using Conclustador**

For each dataset, the table indicates how many clusters were detected by Conclustador. When more than 1 cluster were identified (i.e. cases of incongruence), the value of the CH index is indicated within brackets.

dataset	Conclustador analyses	
	Number of clusters	CH index value
Derelle and Lang 2012	1	/
He <i>et al.</i> 2014	1	/
ALPHAPROT	2	7.6428
EUBAC	2	8.3325

#### ALPHAPROT

##### cluster0

atp1  
atp2  
DLST  
DLDH  
ETFDH  
Hsp78  
HsIVU  
mHsp60  
mHsp70  
nad7  
nad11  
odpA  
tufA  
ndufv1  
SUCLG1  
UQCRC1  
sdh1  
TypA

#### ALPHAPROT

##### cluster1

atp3  
cytc1  
mrl3  
nad8  
ndufv2  
odpB  
PET112  
CLPP  
etfB  
mtif2  
ndufa9  
NifU  
PCCA  
sdh2  
cox11  
grpE  
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ndufaf5  
nsf1  
PCCB  
UQCRFS1

#### EUBAC

##### cluster0

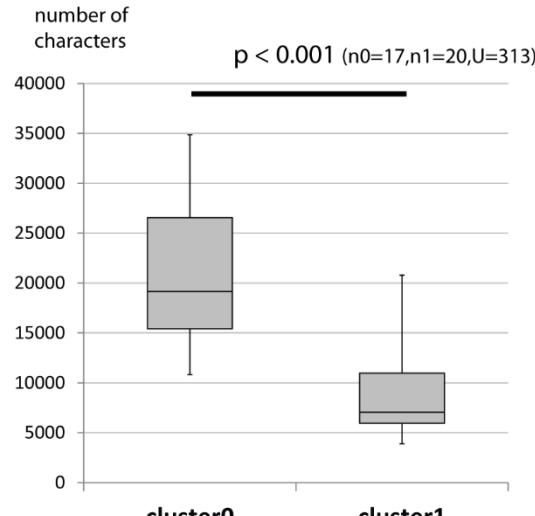
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prot\_2  
prot\_3  
prot\_4  
prot\_5  
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prot\_14  
prot\_15  
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prot\_27  
prot\_23  
prot\_30  
prot\_37

##### EUBAC cluster1

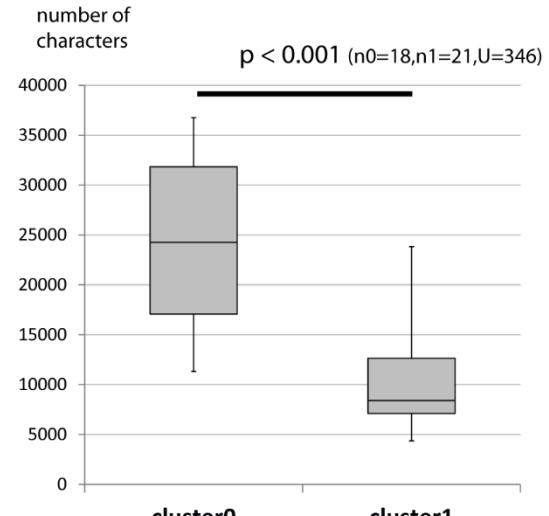
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prot\_32  
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prot\_36

### Distributions of alignment sizes among clusters

The results of the two-tailed Mann-Whitney U-Tests are indicated on top of the box plot graphics.

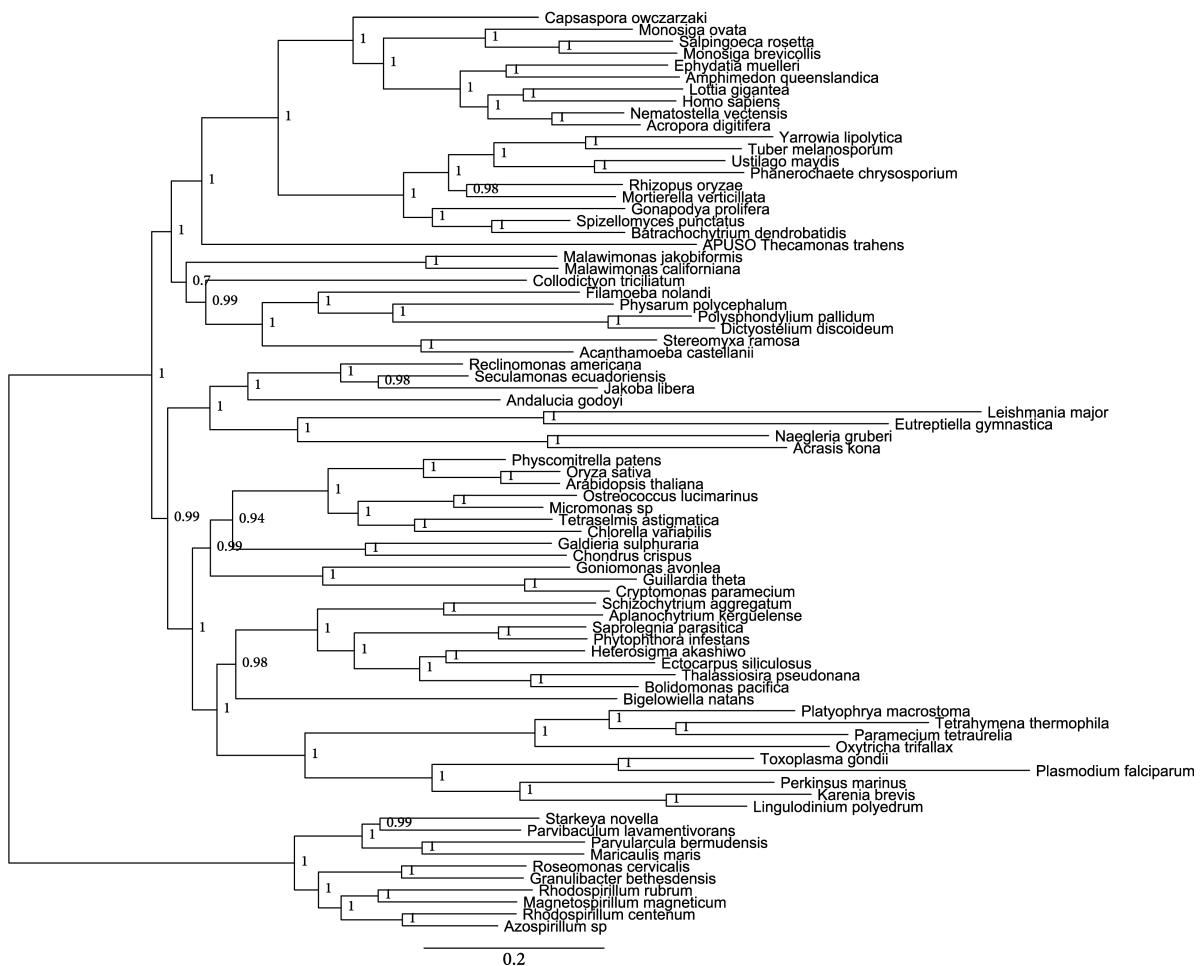


ALPHAPROT dataset

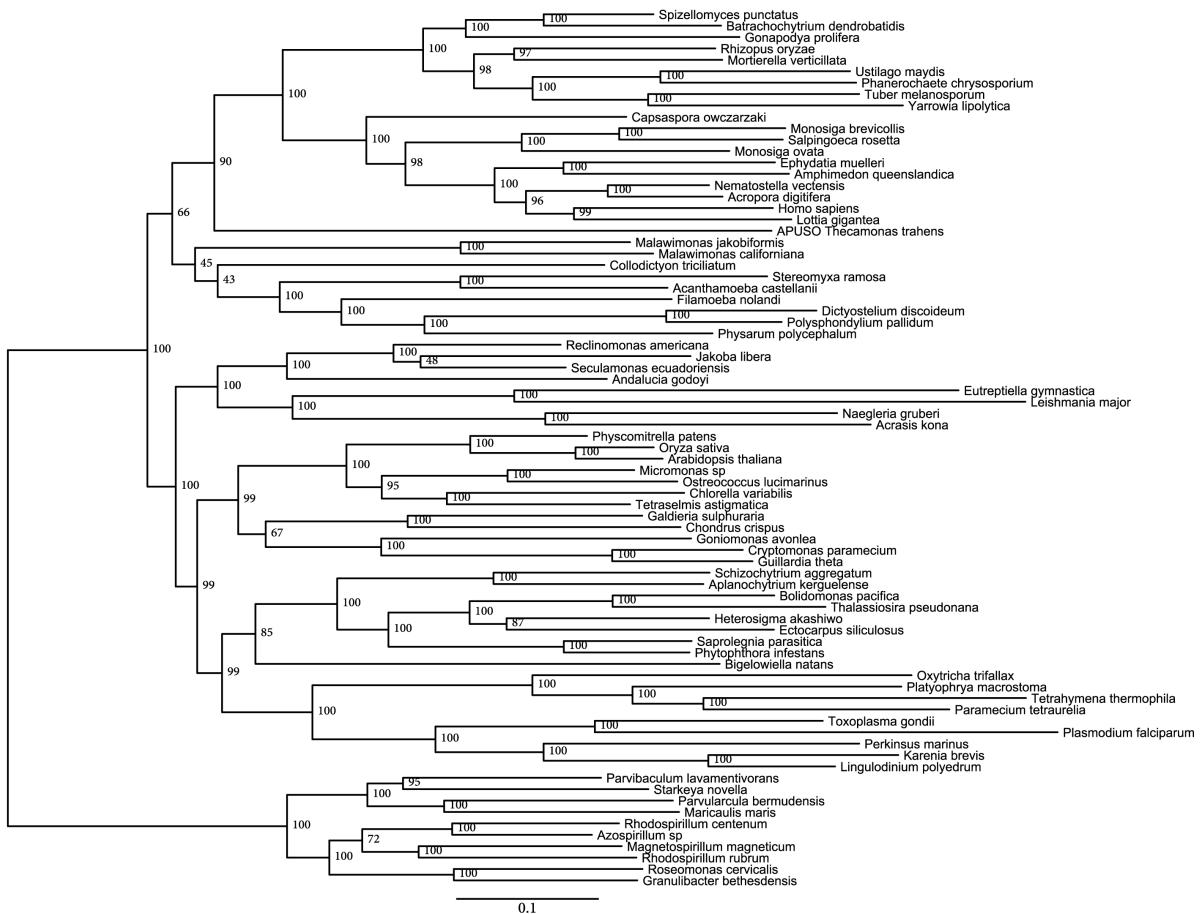


EUBAC dataset

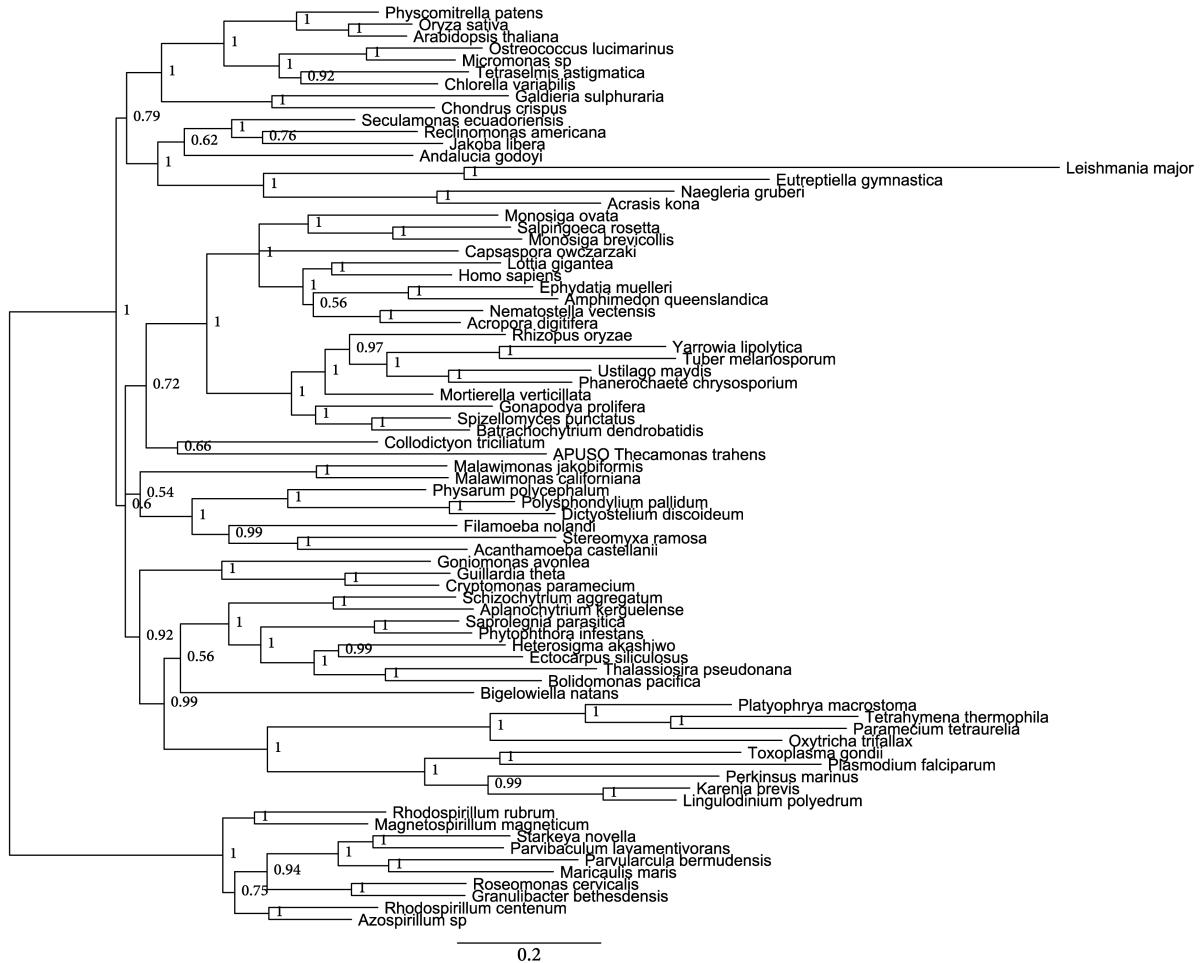
**Consensus Bayesian tree obtained under the CAT model from the ALPHA-PROT cluster0**



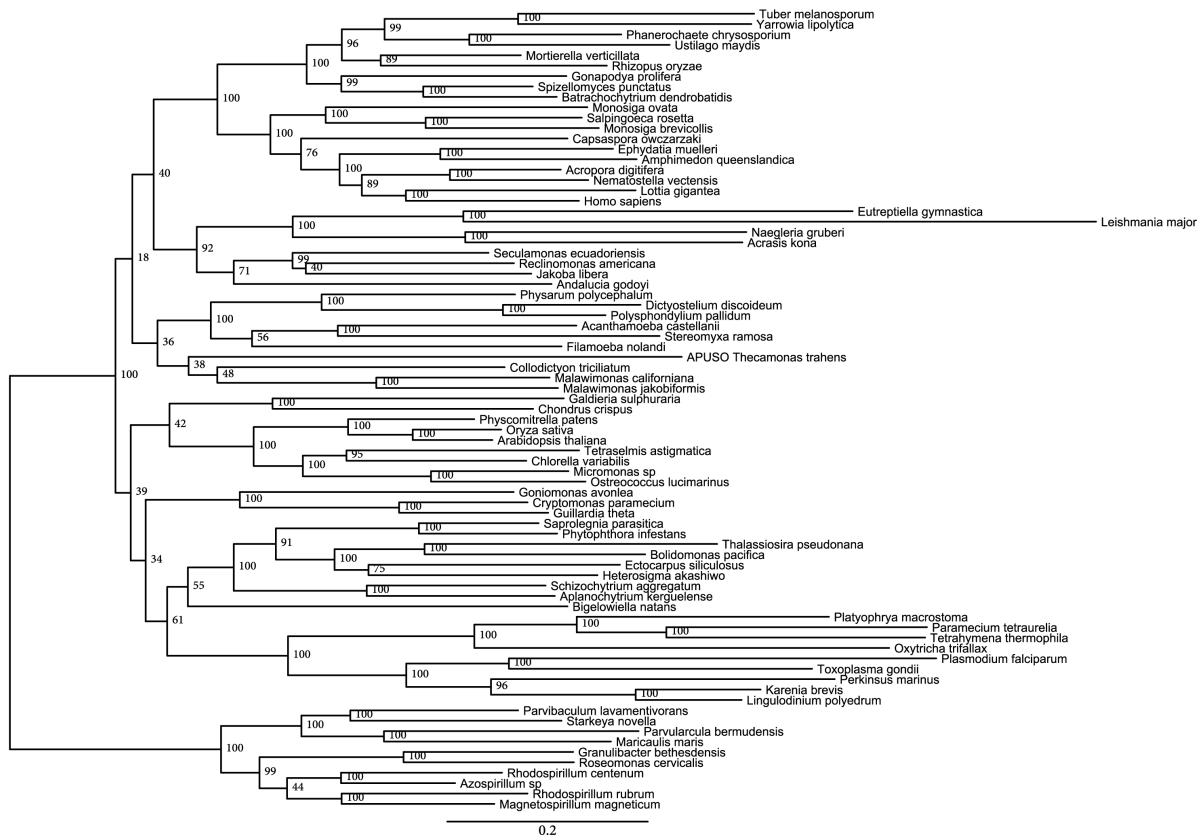
## ML tree obtained under the PROTGAMMAGTR model from the ALPHA-PROT cluster0



**Consensus Bayesian tree obtained under the CAT model from the ALPHA-PROT cluster1**



**ML tree obtained under the PROTGAMMAGTR model from the ALPHA-PROT cluster1**



## **Supplementary data S5: new sequences used in this study**

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>Malawimonas\_californiana@mHsp70

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>Malawimonas\_californiana@nad10

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>Malawimonas\_californiana@PET112

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 VVAEKILA SFN D EATLV N YFKNK ISYVTTKRP IRSLKSF QEA KLEAGA YEF DSDCLTD LYEF YVANSV FQSS VENAT V  
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 >Malawimonas\_jakobiformis@DLDH  
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 VGC IPSK ALL HSS HLYE HAL DAA YGV KIT GV SMD V A QML A QKD NAV K TLT GGI EML FRK N K VQ YV KGF GKL S SA  
 NEV VD M SEGG QSK I RAKN IIIA TG SDV TPL TV PIDE K IV S STG AL S M Q RPK KM V I VGG VIG LE GM GS V W RRL GTE VT  
 VVE YM DRIG AGM DAEIAA QF QRL LT K QGM K FKM STK VM GAE IN SNG AV V N V EA A QGG K P E K L D A C V L V S I G R R PY  
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 MR DDL K D E F L E K H K V K L G F M S G F V R A S A S A L Q A F P V I N A V I D E Q D I V Y R N Y V D I S V A V S T P T G L V V P V L R D V D R M S F A  
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 KDDFKFYD AFSK N L K L G I H E D S A N R A K L A E F L R Y F S S K S A D E M T S L T D Y V T R M K E G Q D Q I Y Y I T G E S R A A V E S S P F L E  
 GLKKRNIEVL FMTD PIDEY A I Q Q L K E F Q G K K L V N I T K E N L N L G E S E E E K K R I E E E K A A F E P L C K T M K E I L E N K V E K V V L G  
 Q R I V E S P C V L V T S E H G W S A N M E R I M K A Q A L R D S S M S M Y M T S K K T M E I N P S H I I K E L K N R V E R G D K A V K D L I M L M Y E T  
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 N R A V N T D G F Q F N L Q T H V L P V L A T V K S E L E N P T G A K H Q A L L K L L A D E L K C D P A V D F D L V L C D T Q P A Q L T G A M E E  
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 LHR NEF S GALT G LTR VRR FC QDDA HIF C TLG QI QA E VKG ALD FIKH VY VGF RFE EL STR P EK AL GT VE QWD KA EKA  
 LAA LDD FGQEW KIN PGD GA F YGP KI DVG FD AL RR HH QC ATI QL DF QLP QR F ELEY GET G KET PV V HRA VLG SLER  
 MIA IL TEH FAG KWP FWV SPQR V RL PKDC MAFA EEV RA AL HD AGY F VD VD ST DDT IQ K KIRNA QVA QV NY ML VVG  
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 DS QL GG V KEY RY FD KTT KGLD FA GLK SDSL A APNG S M VLL HAC HN P TGV DPT QD QW V EIS RL CKD KQH FV FDS AY  
 QGF ASG DPTR DAF A VRQ FV RDG HD VV V A QSF AKN FGL YGERI G FHV TGS DKE A E ILL S QRL I R P M YSN P Q T GARI  
 VET I TDK GLT A HIF C TLG QI QA E VKG ALD FIKH VY VGF RFE EL STR P EK AL GT VE QWD KA EKA  
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DSYAVGIATEVKSMEGAHEEPV PVVSAAA KDAEPVAAA EHENGDEHVFEVVP EWDVDSTL FRAFMLGDESPVVLQ  
MVRATPTGFRIQHIGSNYDVKVM SKLEAKL MPYMPEKKAMDTSKM LLSPMPGALRH VYVNVGDQVTMGQPLAVVE  
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MISR KWLLTVGLEVHARIKAATKL FSGAGT DV SARPNSQVGFFDAAVPGTLPVL NRECVMQAIRTSVALQGHVALRST  
FDRKHYFYHD LPHGFQITQKFTQLMRDGKLHVAEPKGP SKTIGV KYLQ LEVDSGKTIHDLS PQFSYVDLNRAGA ALMEI  
VTEPEIRTPGEAASFVRTLQETRMFDANS GTTRMRKT KesAWD YRFFPEPDLP LLIT QIRQSLP TPEQMAELV AQTGI  
SAADARLIVADPDLSLRFK EASSGRSAKKVANWL ISDLYG LLNQ DDL SVSQCPVTPQQLGSLV DVI ESGSVTVSLGKDII  
HRMFHGDRRTAQDIASAE GMIVVQDEA IRTLC KEIVEANPAEVAKYIAGRKNVFGFFVGLAMKKTN GRANPTLVNA  
ALTEILARQKAAASLAEGRSQ

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LRAVSRVARAVDSVRHASDTAVSTLPNGLRVATEEKLGETATIGVIFIDAGSRYETEQNNGVAHYLEHLAFKGTKTRT  
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DRLHESAYQGTPLGRTILGPEENIRAITRDDL VN FVQTHYTAPR MIVV GAGAVSHQQLTQ LAEKSFGSLPSKAATPISKL  
PCKYTGS DILVQNDDGL THVIAVQGVGW AHDALT LMVMSIVG SWDRNAVGARH VSSK LAGEITTRYDLARSFKS  
FNTCYTDGLFGCYAVXQRVDDH T L DDLV YSIENEW VRL CYDVT EHEVERA KSQLK ASFLM GLD GTT AVVEDIGRQL  
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SSSHGHGHGTVA VVPSKAR RAAGLFG LASGGC VPSF SVGRV DMSRTL TRSFASAAGPESGYEGGEQAT ARSN VGG DP  
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AVPLSELKDPQDDSARTKQPEWL VV LGVCTH LGCV PMAGQGEYGGWFCPC HGSHYDISGRIRKGPA PLN LEVPPYSFV  
DGGAKIRIG